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ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIRST  
ANNUAL MEETING OF THE AMERICAN PHYTOPATHO-  
LOGICAL SOCIETY, COLUMBUS, OHIO, DECEMBER  
27 TO 30, 1939, INCLUSIVE

(NOTE: The arrangement of the abstracts is alphabetical by author or senior author.)

*A New Strain of the Tomato Leaf Mold Fungus Cladosporium fulvum.* L. J. ALEXANDER. Several workers have reported that the tomato species, *Lycopersicon pimpinellifolium*, is highly resistant to the tomato leaf mold fungus, *Cladosporium fulvum*. Recently, the new variety Globelle, developed from crosses between *L. pimpinellifolium* and varieties of the domestic species suddenly became susceptible. Previously, Globelle had shown as high a degree of resistance as *L. pimpinellifolium*. Investigations that followed have shown that the difference in resistance is due to a new strain of the leaf-mold fungus. In cross-inoculation experiments, plants of the varieties Globe and Globelle were uniformly susceptible to spores of the fungus collected from diseased Globella leaves. However, when plants of the two varieties were inoculated with spores collected from diseased Globe leaves, secured from a location remote from where the new strain of the fungus was present, the Globelle plants were highly resistant and the Globe plants susceptible. Inoculation experiments also have shown that *L. pimpinellifolium* is susceptible to the new strain of *C. fulvum*.

*Bacterial Stalk Rot of Field Corn Caused by Phytomonas lapsa, n. sp.* P. A. ARK. Bacterial stalk rot of field corn occurred in epidemic form in several counties in California in 1937. It resulted from the use of contaminated seed or from survival of the organisms in the soil. It is a parenchymatous disease. Under favorable conditions (high humidity and high temperatures) a rapid rotting of the leaves and the stalk occurs, often causing the plants to fall to the ground. The pathogen was isolated from the external parts and from the alimentary organs of *Diabrotica* beetles. The organism is a short bacterium ( $1.55 \mu \times 0.56 \mu$ ), motile by 1 to 4 polar flagella. It produces fluorescence in Ushinsky's, Fermi's, and Cohn's solutions. Acid, but no gas, is produced from dextrose, sucrose, maltose, lactose, glycerine, arabinose, xylose, galactose, raffinose, and mannitol. The name, *Phytomonas lapsa* is proposed, the Latin word *lapsus*, meaning falling, descriptive of one of the symptoms of the disease. The disease is controlled by dusting the seed with Semesan Bel. Of many species of plants inoculated with *Phytomonas lapsa*, n. sp. only sugar cane seedlings developed a disease similar to that of corn.

*Relation of Reducing Substances to Longevity and Virulence of Phytopathogenic Bacteria.* P. A. ARK. Vitamin C (1: 200 to 1: 1,000,000), cysteine (1: 1,000), glutathione (1: 1,000), pyrogallol (1: 1,000), resorecinol (1: 10,000) and tannic acid (1: 100 to 10,000) prolonged the life of *Erwinia amylovora*, *Phytomonas malvacarum*, *Ph. pisi* and *Ph. stewartii*, when these materials were incorporated into potato-dextrose peptone agar on which the pathogens were grown. The higher the concentration of the vitamin C and other reducing substances the longer the bacteria can live on the medium without subculturing. Moreover, this property is retained by the organisms for some time when they are transferred into media without the reducing substances. Thus, *E. amylovora* lived 6 months instead of 2 weeks on the potato-dextrose-peptone agar when transferred to this medium after 2 months on media containing vitamin B (betoxin, 15 mg. per liter of media) or resorecinol (1: 10,000). Virulence of *E. amylovora* diminished when grown without subculturing from 1 to 4 months on the potato dextrose-peptone agar plus the reducing agent. Virulence returns to normal very promptly, even after the first transfer to a medium without any reducing agent or a medium of a very low reduction potential.

*Fusarium Wilt of Cotton and Tobacco Apparently Caused by the Same Organism.* G. M. ARMSTRONG. Isolates of *Fusarium* spp. obtained from wilting cotton and Burley tobacco in South Carolina have been used in cross inoculations on the susceptible varieties Farm Relief (cotton), Burley 5 (tobacco), and the resistant tobacco varieties, Burley 31 and Gold Dollar. A *Fusarium* obtained from wilting tobacco in Kentucky also was used with the tobacco varieties. Inoculations have been conducted in water-cultures in the greenhouse and in pots of soil out of doors. The wilting of the susceptible cotton and tobacco varieties in water-culture after inoculation with the S. C. cotton fungus varied from 87 to 100 per cent and with the S. C. tobacco fungus, 87.8 to 100 per cent. Seventeen per cent of wilting occurred with one resistant variety of tobacco inoculated with the cotton fungus. No external symptoms of wilting occurred in either resistant tobacco variety grown in soil, though the fungus was recovered from some plants inoculated with

each isolate. Wilting of the susceptible variety of tobacco in water culture was slightly more than twice that in soil. These results indicate that *Fusarium vasinfectum* and *F. oxysporum v. nicotianae* are the same. (Cooperative investigations, South Carolina Agricultural Experiment Station and Division of Cotton and Other Fiber Crops and Diseases, U. S. D. A.)

*Studies of Olpidium trifolii and Urophlyctis trifolii on White Clover in Louisiana.* R. E. ATKINSON. Leaf curl of *Trifolium repens*, caused by *Olpidium trifolii*, was found in early February, 1939, in almost every clover patch in the vicinity of Baton Rouge, Louisiana. A month later a gall, caused by *Urophlyctis trifolii*, was observed on the same host in 2 low areas that were inundated for several hours after every heavy spring rain. This is the first report of these 2 organisms in the United States. Ever since the original descriptions of these 2 fungi, there has been considerable uncertainty as to whether or not they were identical. The writer's studies, however, indicate that they are different. *Olpidium trifolii* produces zoosporangia with 1 or more exit tubes. Both resting spores and zoosporangia are characteristically found in the epidermal cells. Resting spores are from 15  $\mu$  to 40  $\mu$  in diameter. Resting spores of *Urophlyctis trifolii*, ranging from 30 to 50  $\mu$  in diameter, are formed in subepidermal chambers by a definite rhizo-mycelium of turbinete cells and slender strands. The so-called haustoria form a crown about the top of the young resting spores. When the spores mature, the "haustoria" disappear, leaving 9 to 15 pits in the external wall, as is true of *Urophlyctis alfaifae*, also.

*Pathogenicity and Hosts of the Fly-Speck Fungus of Apple.* R. C. BAINES. The fly-speck fungus *Microhyriella rubi* has been collected on the following hosts: *Acer saccharum*, *Quercus alba*, *Rubus allegheniensis*, *Sassafras varifolium*, *Salix nigra*, *Smilax hispida*, *Staphylea trifolia*, *Gleditsia triacanthos*, *Khus glabra*, and *Xanthoxylum americanum*. Mature ascocarps, ascii, and ascospores from each host were morphologically similar. Ascospores were found to mature during the first part of June at Lafayette, Indiana. The fungus was isolated in single-spore cultures from the first 7 hosts listed above. Cultures from all 7 appeared very similar on potato dextrose agar. The fungus grows slowly and produces a grey, compact colony on most agar media. Growth was obtained between pH 1.8 and 8.2 on oatmeal agar. Growth occurred over the temperature range, 5–27° C., best growth appearing between 15–24° C. Attempts to induce sporulation in culture failed. Immature apple fruits were inoculated with mycelium from cultures isolated from the first 5 above-named hosts. Typical fruiting bodies and symptoms of fly speck were obtained in each case. The fungus differs markedly in symptoms produced on apple and in cultural characteristics from the sooty blotch fungus *Glocodes pomigena*.

*Acquisition and Transmission of Viruses by Dodder (*Cuscuta subinclusa*).* C. W. BENNETT. Dodder (*Cuscuta subinclusa*), growing on curly-top-infected beet or Turkish tobacco plants appears to acquire concentrations of virus equal to those of the host plant. When stems of dodder were trained from diseased beets and established on healthy beet or Turkish tobacco plants the curly-top virus was transmitted to from 2 to 5 per cent of the plants. Where no transmission occurred the virus was lost from the dodder within a few days following breaking of connection with infected plants. Dodder became infected with cucumber mosaic virus, retained the virus indefinitely on immune plants, and transmitted it to more than 90 per cent of Turkish tobacco and *Nicotiana glauca* plants on which infected stems became established. No virus was obtained from juice of dodder growing on beet plants affected with beet mosaic or from juice of dodder growing on Turkish tobacco plants affected with common tobacco mosaic. By means of dodder the virus of cucumber mosaic is easily separated from a mixture of cucumber-mosaic virus and tobacco-mosaic virus in Turkish tobacco. Transmission of the virus of cucumber mosaic from Turkish tobacco to Turkish tobacco and *N. glauca* was obtained also, using *C. californica*.

*Mechanical Transmission of Aster-yellows Virus to Leaf Hoppers.* L. M. BLACK. The aster-yellows virus has been mechanically transferred from viruliferous to non-viruliferous aster leaf hoppers (*Macrostelus divisus*) at 0° C. Dilutions of juices from macerated viruliferous leaf hoppers as high as 1: 100 in 0.85 per cent NaCl solutions are infectious when injected into nonviruliferous leaf hoppers by means of capillary glass tubes. As high as 40 per cent of the inoculated insects become infective for plants after an incubation period of from 2 to 6 weeks. Leaf hoppers, infected mechanically, appear to transmit virus to healthy aster plants as efficiently as insects that become viruliferous through feeding on diseased plants. The successful mechanical transfer of virus from insect to insect has made possible a study of virus multiplication in this vector. Non-viruliferous leaf hoppers were fed 2 days on diseased aster plants and then maintained on rye, which is immune from aster yellows. No virus could be detected in such insects

during the first few days after they had fed on diseased asters. Later, however, juice from such leaf hoppers was infectious at dilutions up to 1: 100 when injected into non-viruliferous insects. This is considered good evidence of multiplication of aster-yellows virus in its insect vector.

*Cytological Studies of Sporidial Fusion in Ustilago zeae.* DONALD H. BOWMAN. Sporidial fusion in paired monosporidial cultures originating from single chlamydospores of *Ustilago zeae* was found to occur readily, although not abundantly, in cultures of very low nutritive value after a period of 15 to 20 hours, depending upon the temperature at which the cultures were incubated. Initiation of the dikaryophase did not occur in any fixed manner, but was effected by conjugation of 2 compatible haploid cells, either sporidia or cells of 2 haploid hyphae resulting from sporidia. Nuclei from the haploid cells became paired in a fusion cell, which gave rise to the dikaryotic hypha. Such hyphae continued growth throughout the experiment without reverting to the production of sporidia. The end cells of the dikaryotic hyphae absorbed and retained the various dyes used much more readily than did the older portions of the hyphae and the sporidia. Apical cells of these hyphae uniformly contained one pair of nuclei each. The older cells contained well-defined nuclei either singly or in pairs or were partly or entirely devoid of cell contents. Cells of fused sporidia frequently contained a single nucleus or were either partly or entirely empty.

*The Boron Deficiency Disease of Apple.* A. B. BURRELL AND F. H. LEWIS. Numerous terms for the different symptoms are in use. Confusion would be avoided if all but the following were eliminated: internal and external cork of fruit; incipient dieback, dieback, rosette, (and possibly internal bark necrosis). Drought indirectly was responsible for a 1939 outbreak of cork in New York and New England. Borax applied to the soil gave control in the 3 major apple-producing regions of New York. Borax soil applications, in the spring of 1937, were still effective throughout 1939. Part of the boric acid injections made in late summer of 1936 had become ineffective by 1939. A borax soil application on June 30 when some cork already showed, largely prevented late-season development, which was abundant in cheeks. The effectiveness of borax added to orchard sprays remains in doubt. Trees treated with Chilean nitrate of soda have not shown significantly less cork than those receiving other nitrogen carriers. Six annual applications of manure did not prevent internal cork. Extreme soil acidification with sulphur applied from 1927 through 1937 failed to give cork control in 1939. In commercial practice, minor boron foliage injury occasionally results from excessive soil application to young replants and from careless concentrated application near tree trunks.

*Sulphur as a Protectant of Cereal Crops.* KARL D. BUTLER. Sulphur dusting of cereal crops on a field scale has been carried on in New York state for the past 3 seasons. Through the use of a power duster mounted on a pick-up truck and equipped with a 40-foot boom, effective protection has been obtained. Epiphytotics of *Puccinia rubigo-vera* *tritici*, *P. coronata* and *Erysiphe graminis* *hordei* have been checked with 2 to 5 applications of sulphur at the rate of 30 pounds an acre per application. In 1938 and 1939 satisfactory protection of wheat from *P. rubigo-vera* *tritici* was accomplished with but 2 well-timed applications. Timing of applications was facilitated by studies on the dissemination of urediospores by means of weather-vane spore traps. Increased yields in bushels per acre ranged up to 10.45 of wheat, 25.53 of oats and 5.36 of barley in 1937; 12.56 of wheat, 28.81 of oats, and 10.23 of barley in 1938; and 8.38 of wheat, 6.90 of oats, and 6.85 of barley in 1939. Reduced yields due to injury caused by the equipment in wheat fields were found to be slightly less than one bushel per acre.

*Cross Inoculations with Loose Smut of Wheat.* RALPH M. CALDWELL AND L. E. COMPTON. Seven collections of loose smut, *Ustilago tritici*, obtained from individual soft wheat varieties in Indiana, Ohio, and Illinois, in 1938, were tested for pathogenicity on 7 soft wheats, (Kanred-Gipsy, Purdue No. 4, Nabob, Forward, Trumbull, Hussar, Kawvale) showing field resistance, and 2 (Wabash, Purdue No. 1) showing high field susceptibility. Inoculations, by the part-vacuum technique, produced over 75 per cent infection in highly susceptible varieties. Evidence was secured of a high degree of host specialization of the loose-smut fungus as it occurs on soft wheats in this region. The field-susceptible varieties, Wabash and Purdue No. 1, were heavily infected when inoculated with spores from the same 2 varieties, respectively. However, in cases where either variety was inoculated with smut from the other variety, a high degree of resistance, or immunity, was demonstrated. Kanred-Gipsy, a variety previously found resistant to both natural and artificial infection, proved highly susceptible to 1 collection from Ohio. Purdue No. 4 proved highly resistant to certain collections but susceptible to others. Kawvale and Trumbull were resistant to all collections, while Nabob, Forward, and Hussar, each, produced a few infected plants from certain collections. (Department of Botany, Purdue University)

University Agricultural Experiment Station, Lafayette, Indiana, and Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

*The Effect of a Diseased Cotton Seed on an Adjacent Healthy Seed.* K. STARR CHESTER AND GERTRUDE TENNYSON. The question of whether a diseased cotton seed jeopardizes the success of adjacent healthy seed was tested by direct observation of the success of healthy seed in the presence of diseased seed under greenhouse conditions and by analysis of greenhouse and field data in the light of the mathematical probability of successful development of healthy seed alone or in the presence of diseased seed. The diseased seeds were infested with various amounts of *Glonospora gossypii*, *Fusarium moniliiforme*, *Sclerotium bataticola*, *Bact. malvacarum*, and other organisms. The results indicated that the stated hazard is slight or negligible under a variety of environmental conditions, implying that the beneficial effect of "Cercsan" is principally on the treated seed itself, and that external factors, principally *Rhizoctonia*, are of much greater importance in influencing the success of seedlings than infestation among the seed.

*Disease Resistance in the Genus Nicotiana.* E. E. CLAYTON AND H. H. FOSTER. Over 1,000 collections of *N. tabacum* ( $n=24$ ) from all known areas of occurrence have been tested for resistance to blue mold (*Peronospora tabacina*), black root rot (*Thielaviopsis basicola*), wildfire (*Bacterium tabacum*), bacterial wilt (*Bacterium solanacearum*), root knot (*Heterodera marionii*), and mosaic. A high degree of resistance was found for black root rot and mosaic, but only moderate to slight resistance for root knot, wildfire, blue mold, and wilt. Wilt, root-knot, and blue-mold resistance was recessive and conditioned by multiple factors. Some 30 other *Nicotiana* species have been studied with respect to disease resistance. Some of the more promising of these are, *N. debneyi* ( $n=24$ ), which appears immune from blue mold and black root rot, and resistant to wildfire; *N. glauca* ( $n=12$ ) immune from black root rot and highly resistant to mosaic, root knot and wildfire; *N. repanda* ( $n=24$ ) highly resistant to wildfire, black root rot, and root knot. Smith's allo-polyplloid (*N. tabacum*  $\times$  *N. glauca* -  $n=36$ ) shows resistance to root rot and root knot. These, and other species of *Nicotiana*, offer a reserve of resistance approaching immunity for all diseases considered, excepting bacterial wilt.

*Sweet-potato-storage House Fumigation.* HAROLD T. COOK AND T. J. NUGENT. Formaldehyde, a formaldehyde-sulphur dioxide mixture, chloropicrin, ammonium hydroxide, carbon tetrachloride, and ethylene dichloride were compared as fumigants for sweet-potato-storage houses. The materials were tested on spores from pure cultures of *Rhizopus nigricans* and *Ceratostomella fimbriata* applied to wooden blocks, suspended in 1-liter flasks. After the fumigants were added the flasks were sealed with glue-coated paper for 24 hours. The quantity of formaldehyde usually recommended (3 pt. to 1000 cu. ft.) was found to be about 4 times the amount needed for sterilization. Fumigation tests for shorter periods indicated that the fungi were killed within 2 hours. Larger quantities of the fumigants were required for sterilization when the storage chambers were partly filled with pieces of wood. Chloropicrin was effective against both fungi, even at concentrations as low as 1/16 lb. in a moist atmosphere, but was only partly effective, even at higher concentrations, in a dry atmosphere. Ammonium hydroxide and the formaldehyde-sulphur dioxide mixture were not so effective at low concentrations as were formaldehyde and chloropicrin. Carbon tetrachloride and ethylene dichloride were entirely ineffective against *Rhizopus*, even when 12 pints to 1000 cubic feet were used.

*Preliminary Serological Studies of Phymatotrichum omnivorum.* R. W. CUMLEY AND G. W. GOLDSMITH. A preliminary serological study was carried out to determine the relationship of the cotton root rot fungus, *Phymatotrichum omnivorum*, to the various members of the different groups of fungi. Extracts of freshly collected or cultured species were compared with the root-rot fungus in the precipitin and the complement fixation tests. Results indicate that the cotton root-rot fungus serologically is more closely related to the various Gasteromycetes than to the other fungi tested.

*Organic Seed Protectants for Lima Beans.* H. S. CUNNINGHAM AND E. G. SHARVILLE. Damping-off in most seasons constitutes a major problem in the chief Lima-bean growing areas of the United States. No chemical seed treatment has yet been found that is entirely suited to Lima beans. Organic mercurials usually stunt the plants throughout their life-time, while copper oxides in many instances harden the seed coat, making it difficult for the plumule to break out. Preliminary laboratory and greenhouse experiments indicated that 2 synthetic organic chemicals developed by the Crop Protection Institute were efficient, noninjurious seed protectants for combating damping-off of Lima beans. The laboratory and greenhouse findings were substantiated by field experiments conducted on Long Island during the past season, where seed treatment with the new materials No. 120, No. 98, and New Improved Semesan Jr. in that order resulted in

significant reduction of seed decay of the Lima beans. Graphite seed treatment reduced drilling friction, and that in turn appeared to reduce cracking of seeds and subsequent decay in the soil.

*A Method for Testing the Pathogenicity of Actinomyces Isolates.* PHARES DECKER. Plants were grown from scab-free, cold-formaldehyde-treated tubers in sterilized silt-loam soil in 6-inch clay pots, each fitted with a 6-inch clay saucer. The isolates were increased on potato-dextrose agar in Petri dishes and washed from the plates into the sterilized soil over the seed piece and covered 2 inches deep. The greenhouse, equipped with a painted concrete floor and metal-leg benches with wooden tops, was fumigated with formaldehyde gas and sterilized with a 2 per cent solution of carbolic acid before the pots were moved in the house. The water was applied to the pots by means of the 6-inch clay saucer placed under each pot to avoid overhead splashing. During the past year, of the 225 isolates tested, each replicated 4 times, 35 were strongly pathogenic, 43 were weakly pathogenic, while 157 were nonpathogenic under the conditions tested. The tubers from 300 control pots placed at random among the pots containing the scab inoculum were absolutely scab-free.

*The Relation of Stomata to Wildfire Infection.* STEPHEN DIACHUN. The amount of wildfire on inoculated leaves of greenhouse or field-grown tobacco plants was found to be dependent on the stomatal condition of the leaves at the time of inoculation. During the day stomata are usually open and tender leaves of vigorous plants atomized with a bacterial suspension become severely infected if the nozzle of the atomizer is held about 2 inches from the lower surface of the leaves. At night or in artificial darkness stomata are usually closed or nearly so; they also are apt to be closed during the day on leaves that are wilted, excessively shaded, or turned up so that the lower surface is exposed to the sun. Under any of these conditions, when stomata are closed, leaves atomized with *Bacterium tabacum* develop little infection. During some rainstorms stomata were open, and during others they were closed, depending perhaps on the light intensity. It is suggested that natural infection by bacteria carried in wind-blown rain may occur only when stomata are open.

*Inoculation Experiments and Reaction of Inbred Lines of Corn to *Ustilago Zeae*.* JAMES G. DICKSON AND DONALD H. BOWMAN. Inoculation studies with *Ustilago zeae* have been conducted during the past 2 seasons. A spore suspension in 0.8 per cent solution of rosin-fish-oil spray soap appeared applicable to large-scale inoculation experiments and gave good differentiation for smut reaction on inbred lines of corn. Six such lines, differing in resistance to smut, were inoculated at 4 stages of plant development in 1939. The soap solution alone increased smut infection an average of 10.2 per cent over the controls. The suspension of chlamydospores in the soap solution increased smut infection an average of 39.6 per cent over the controls. The range in averages for the individual lines was from 11.2 to 69.7 per cent increase over the controls. The smut reaction of the individual lines in the inoculation experiments for the 2 years was in fair agreement with that resulting from natural infection. (Cooperative investigation between the Wisconsin Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

*Modifications in Cells of Plants Affected by Virus.* JEAN DUFRENOY. I. The inhibition of terminal growth at root tips of sugar beet affected by *Jaunisse* (a virus disease in northern France and Belgium) results in many mitochondria differentiating into "starch-storing" amyloplasts in postmeristematic cells, where homologous cells of sound beets show only rod-like "inactive" mitochondria. The same response has been observed in beets affected with curly top.

II. Enhanced respiration in virus-affected cells may shift the pH of the vacuolar solution upwards, as organic acids are oxidized to CO<sub>2</sub>. The lowering of the acidity, together with altered viscosity resulting from accumulation of tannin in the vacuolar solution, causes calcium oxalate to crystallize as large tetrahedric crystals, often clumped together as, for example, in cells of Up-to-Date potato leaves, along the necrotic perivascular lesions caused by Virus Y.

III. Chloroplasts in affected leaves are more apt to swell, when sections of fresh tissues are immersed in hypotonic solutions: chlorophyll may migrate away from swollen chloroplasts, to be absorbed in oil inclusions.

*Reactions of Cells of Sugarcane Stalks to the Red Rot Fungus, *Colletotrichum falcatum* Went.* JEAN DUFRENOY AND C. W. EDGERTON. Following inoculation, spores lodge in the pitted vessels and germinate. Mycelium grows through pits into the living cells of the parenchyma and comes into close contact with the nuclei. Vacuolation is induced and numerous oil droplets along the cytoplasmic strands outline the vacuoles.

In susceptible varieties such as C.P. 807, more cells become heavily infected as the infected cells die and the hyphae are outlined by rows of droplets of vacuolar material showing staining reactions of phenolic compounds. In resistant varieties such as Co. 281 phenolic compounds are evident throughout the vacuolar system of infected cells and the mycelium becomes imbedded in the reactive products of the cell. The red pigment produced by the infected tissues diffuses out into the adjoining cells and is absorbed by the lignified spirals of the vessels. From sections of red-rot lesions, treated with 0.25 per cent NaOH, a yellow solution is obtained, which oxidizes and turns brown on contact with the air.

*The Influence of Ultraviolet Irradiation on the Pathogenicity of *Phytomonas tumefaciens*.* B. M. DUGGAR AND A. J. RIKER. In this work monochromatic ultraviolet of wave length 2650 Å was employed, and suspensions of the bacteria were exposed for a time to intensities sufficient to give about 99 per cent killing, i.e., 1 percentage survival. Four strains of the organism were selected; one of these characteristically virulent (gall-producing), one attenuated by chemical treatment, and two strains partially attenuated by the same type of treatment. Of these two last, one was losing and one recovering gall-producing capacity. Gall production of all treated and control suspensions was tested by inoculation into young tomato plants, with periodic observation and measurement of the gall number and size. About 50 colony isolates of each exposure were used, and for each isolate 10 punctures were made; the control was similarly used. The present status of the results point to the following: Neither was the virulent strain nor the avirulent one materially affected in pathogenicity by irradiation. The partly virulent strains, on the whole, gain in pathogenicity as a result of irradiation. The survivors of irradiation-treated isolates exhibit a greater range of variability than the corresponding controls.

*Root Girdle Caused by High Wind.* E. L. FELIX. Root girdle, characterized by marked constriction of the primary root near the soil surface, frequently causes heavy losses in small seedlings of beets, carrots, lettuce, and spinach in Western New York, especially on muck soil. In lettuce, root girdle (previously incorrectly called stem-girdle) commonly destroys plants one-fourth or more grown. Lettuce suddenly turns yellow, wilts, easily breaks off at the constriction, and is sometimes mistaken for cutworm injury. Reddish to reddish brown vascular discoloration sometimes originates below the girdle and extends upward to it, seldom above. Leaves of girdled lettuce sometimes are inclined in one direction and the roots in the opposite, presenting a one-sided appearance. That high wind is the primary cause of root girdle is indicated by observations over a 15-year period. Root girdle occurs only following high wind, usually 2 to 4 days later, and in wind-swept portions of the field. It appears and disappears in large part suddenly and simultaneously. Occurrence is of short duration, the majority of affected plants dying in 7 to 10 days. Leaf inclination in girdled lettuce tends to be leeward. Associated bacteria and *Botrytis* fail to reproduce typical root girdle and are secondary invaders. The manner of girdling appears to be mechanical injury, desiccation, and shrinkage of tissues.

*Variation in *Dothiorella ulmi*, the Elm Cephalosporium.* LAWRENCE M. FENNER. Variation in cultural characters in the elm die-back organism, *Dothiorella ulmi*, has been observed. Sixteen isolates from widely distributed points in the eastern United States have been studied by single-spore methods and sector subcultures. Three phenotypic groups, A, B, and C, were noted. The range of variation was greatest in group A. A few forms have been considered relatively stable. Certain phenotypes are reported as new and undescribed in the literature. In a study of more than 2,000 single-spore colonies and their numerous subcultures, a close relationship was recognized among the phenotypes of the 3 groups.

*The Thixotropic Character of the Tobacco-Mosaic-Virus Protein.* VERNON L. FRAMPTON. Three hundred feet of 16 mm. Kodachrom photographed through crossed polaroid plates. Interesting features include:

1. An indication of the sol-gel transformation time.
2. The solid character of the gel depicted in a rotating test tube. By way of contrast, debris suspended in water does not rotate with tube, whereas the virus protein gel does.
3. The evidence that the Brownian movement is greatly curtailed is seen in the persistence of the orientation of the anisodimensional aggregates—an orientation induced by a sphere falling through the gel.
4. Vortex motion in true solution is contrasted with the behavior of the virus protein gel under comparable conditions.

5. Demonstration of the gel character of the protein-water system by metathesis of BaSO<sub>4</sub> in the form of what appears a solid vertical rod in the center of the gel.
6. The swastika-shape cross of isocline.
7. The spontaneous repair of structure broken by forced flow.
8. The elastic character of the gel as shown by the very strongly damped harmonic motion of the gel mass as it comes to rest after having been disturbed.
9. A comparative study of the viscosity and diffusion of the protein-water system and of glycine.

*Some Factors that Affect the Spray Program in the Control of Cedar-Apple Rust Fungi on the Apple.* J. M. HAMILTON AND L. O. WEAVER. Studies on potted apple trees in a controlled environmental set-up indicate that fungicides are effective only against infection from the sporidia of the cedar-rust fungi over the area actually covered. Liquid lime-sulphur 1-50 is effective when applied 8 hours after the sporidia are *in situ*, but the wettable sulphur do not inhibit infection at 4 hours. Foliage infection takes place in 6, 4, 2, and 5 hours at temperatures of 9, 13, 17, and 21 degrees C., respectively, but not at 25°. Sporidia may remain viable on foliage without moisture for 24 hours; but there is a marked reduction in the amount of infection after 4 hours. Experiments conducted on dwarf Wealthy and Delicious trees with the apple and quince rusts suggest that fruit parts may be susceptible any time after the opening of the fruit cluster. Prevention of sepal infection is important. The quince rust fungus causes heavy fruit drop, not usually apparent when infection takes place during or soon after bloom. The fruit is most heavily infected at 4 to 10 mm. diam., but it is susceptible at 52 mm. Fruit infection has been obtained in 5 and 12 hours at 16° C. with both rusts.

*Methods for Determining the Effectiveness of Fungicides Against Apple Scab and the Cedar-Apple Rust Fungi.* J. M. HAMILTON AND L. O. WEAVER. The technique of spraying fungicides on potted apple trees, washing them on a turn table, and testing the fungicidal residue by subjecting to an infection period against the spores of *Venturia inaequalis* in a temperature-humidity controlled chamber has been improved upon. Regulated spraying of trees on the turn table, together with modified sulphur and copper analytical methods, has made possible accurate evaluation of fungicides and a more valuable interpretation of data from analyses of fungicidal residues taken in the field. The use of the sporidia of *Gymnosporangium juniperi-virginianae* is preferable to the spores of *V. inaequalis*. A precision laboratory sprayer—a material but limited aid in preliminary fungicidal studies—has been adopted, with the above set-up, for making exact depositions of fungicides or spore suspensions on foliage. Half of each leaf can be sprayed and the other half left as a check. The initial deposit is most accurate and can be tested with or without washing. For example, 30 to 40 gamma of sulphur per sq. in. are required to give protection against sporidia of *Gymnosporangium juniperi-virginianae*, whether it is an initial deposit or residue obtained by washing.

*The Dissemination of Yellow Dwarf of Potatoes and Its Leaf Hopper Vector, Accrata-gallia sanguinolenta.* E. D. HANSING AND VERNON L. FRAMPTON. The incidence of yellow dwarf in potato fields indicates that its leaf-hopper vector migrates in obedience to the laws of diffusion. Data obtained from samples of tubers taken at various distances from adjacent meadows in 2 fields of the variety Rural, and indexed in the greenhouse,

fit the curve  $-\log \frac{I}{I_0} = Rd$  where I is the percentage of infection at the distance d from the meadow,  $I_0$  is the "saturation value" in per cent at the edge of the field, and R is a constant characteristic for the particular observation and represents a composite of various factors, such as population density, proportion of viruliferous insects, prevailing winds, food supply, etc. Values for  $\log I_0$  and for R were 1.78 and .019, respectively, in the one case and 1.32 and .015 in the other. Conclusions that seem reasonable on the basis of these data are: (1) The migration of the insects parallels that of a diffusing substance; (2) The principal vector occurs naturally outside the field in question; (3) The spread from potatoes infected during the current season is a minor factor in the dissemination of the disease.

*Insects in Relation to Root Rot and Basal Stem Rot of Cereals.* E. W. HANSON AND J. J. CHRISTENSEN. Studies during recent years have shown that several species of insects, most of which are not yet definitely identified, attack the basal parts of wheat, barley, and several wild grasses, furnishing avenues of entrance for fungi, in some cases filling the culms with frass, which is a good medium for rapid growth of fungi, and predisposing the plants to root rot and basal stem rot. Infestation of this type has been common throughout the spring-wheat area for several years, the amount varying with the locality and year. In 1938 and 1939, from 70 to 90 per cent of the plants of certain varieties were infested. The durum variety Mindum was extremely susceptible, bread

wheats were moderately susceptible, and oats was virtually free from infestation. Many grasses, including *Agropyron repens*, *A. smithii*, *A. cristatum*, *Bromus inermis*, and *Echinochloa crusgalli* also were infested and predisposed to rots. *Helminthosporium spp.* and *Fusarium spp.* were isolated most commonly. The recognition, selection, and development of varieties resistant to root and foot rot must take into consideration susceptibility or resistance to the predisposing insects and the interrelationships between them and the rots. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

*Effect of Fertilizers on the Development of Bunt of Wheat.* E. W. HANSON AND I. W. TERVET. It has been difficult to obtain consistently satisfactory infection with bunt at University Farm, St. Paul, Minnesota. Therefore, a study was made of factors affecting its development. As fertilizers have been reported to be important, potash, ammonium phosphate, ammonium sulphate, cyanamid, acid phosphate, and urea were added to the soil separately at the rates of 250, 200, 200, 150, and 130 lb. per A., respectively, in 2 applications—the first, 10 days after planting and the second, 18 days later. Seed of 2 varieties, Ceres and Thatcher, was inoculated with a composite sample of chlamydospores of several physiologic races and collections of *Tilletia levis* and *T. tritici*. The experiment included 12 replications in 1938 and 4 in 1939. The relative amount of bunt was high (41.6 per cent on Ceres) in 1938 and very low in 1939 (3 per cent on Ceres), but in neither year was there any correlation between the amount of bunt and fertilizers applied. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

*A Fungous Parasite of the Pine Bark Beetle.* J. G. HARRAR AND J. G. MARTLAND. During the spring of 1939, collections of bark of *Pinus echinata* from eastern Virginia were found infested with the larvae of the pine bark beetle, *Dendroctonus frontalis*, many of which were dead or dying from what appeared to be the action of some parasite. Upon examination, large numbers of the affected larvae were found infested with both fungi and nematodes. Larvae from a number of collections were plated out, and a similar fungus was isolated from each of several larvae from the different collections. A study of monosporous cultures of the fungus has resulted in its tentative identification as a species of *Beauveria*. Results of subsequent experiments have shown that this organism may live as an insect parasite.

*A Wilt of Tree Paeonia (Paeonia montana).* M. R. HARRIS. A fungus disease of tree paeonia has been observed in the San Francisco Bay region since 1932. On old plants it causes a wilting and sudden killing of the current season's growth. The first evidence of the disease is a water-soaked lesion that rapidly turns a light brown, encircles the stem, and causes the growth above the lesion to wilt and collapse. Losses are most severe during the flowering season. If allowed to go unchecked young shoots wilt successively as they appear and the plant eventually dies. Isolations and pathogenicity studies have shown that 2 species of *Coniothyrium* cause the disease. One of these is believed to be *Coniothyrium fuckelii*. Cross inoculations or roses have shown that both fungi can parasitize roses and paeonias. Histological studies show that the fungi are largely confined to the cortical region of the stems. Effective control in a commercial nursery has been accomplished by pruning out infected stems and spraying with ammoniacal copper carbonate.

*Transformation of Haustoria and Hyphal Tips of Puccinia graminis tritici into Spore-like Bodies.* HELEN HART AND J. LEWIS ALLISON. Haustoria of certain races of *Puccinia graminis tritici* may be transformed into urediospore- or teliospore-like bodies within host cells and the hyphal tips of the intercellular mycelium may develop into teliospore-like bodies at a temperature of 29° C., or higher, in seedlings of certain wheat varieties. External evidence of the transformation is the browning of host tissues surrounding the rust pustule, where chlorosis is the normal appearance. In severe cases the browning is marked and sporulation in the pustules is greatly reduced. High atmospheric humidity hastens and accentuates the browning at high temperatures. This seemingly abortive sporulation is common in many collections of race 34 and in certain collections of races 11 and 15, and it may occur in other stem rust races. It is most severe on Kota, Ceres, Kubanka, Aeme, Arnautka, and Thatcher wheats, on Vernal emmer, and on einkorn. It rarely or never occurs on Marquis, Little Club, Mindum, or Spelmar wheats.

*Effect of Soil Reaction on Soil Rot of Sweet Potatoes.* JOHN D. HARTMAN AND R. W. SAMSON. Sweet-potato soil rot was reduced by the use of sulphur and increased by the use of lime on Princeton and Elk sands having initial reactions of around pH 6.3 to

pH 6.9, as measured by the relative numbers and weights of marketable potatoes with no lesions and with small, medium, or large lesions. Increasing the acidity to about pH 5.9 or pH 6 greatly reduced soil rot and decreased yield little or not at all. Soil rot was more prevalent at pH 7.5 or higher and yields were lower. Among replicated plots adjusted to a given pH level soil rot tended to be more severe on those plots, which previous to adjustment had had the highest pH. The above pH determinations were based on samples taken in the spring.

*Association of *Bacterium Phaseoli* and the Virus of Common Bean Mosaic.* FLORENCE HEDGES. *Bacterium phaseoli* has been isolated from bean plants inoculated with bean-mosaic virus, but showing a high percentage of bacterial infection on the inoculated primary leaves. Isolations were made from such primaries and also from trifoliates showing virus infection but no bacterial lesions. Cultures of the bacteria from each source produced 75 per cent typical mosaic and also bacterial infection on healthy bean seedlings 11 days and 6 weeks, respectively, after isolation. The bean-mosaic virus is ordinarily extremely "short-lived" outside the plant. In inoculations, both with a mixture of virus and bacterial cultures and in two parallel sets of serial passages of infected juice therefrom, begun September, 1936, and still in progress, dominant virus symptoms and rather inconspicuous bacterial ones with an occasional passage showing severe bacterial infection appeared to be the rule. In the serial passages, however, after 25 months, the bacterial symptoms suddenly disappeared for 10 successive passages. Shortly after this disappearance, there arose with the 38th serial passage (both sets) an ultra-severe mosaic still persisting (50th serial passage). Bacterial symptoms reappeared. Dissociation experiments are in progress with *Bact. phaseoli* from Lima-pod and 2 highly virulent isolations after 2 years of serial passages. Comparative studies are being made of "atypical" forms arising therein and those appearing in the association studies.

*Spraying Tomatoes for Disease Control.* R. G. HENDERSON AND S. A. WINGARD. During the season of 1939, 22 varieties of tomatoes were sprayed with 3 spray preparations—yellow copper oxide (54Y), red copper oxide with cottonseed-oil emulsion, and a commercial preparation of red copper oxide combined with an emulsified oil (Fugrocide). *Septoria* leaf spot, *Alternaria* blight, and *Phytophthora* late blight occurred generally on all the varieties and were especially severe on the nonsprayed plants. The average yields per acre of all 22 varieties on the sprayed and nonsprayed plots were: yellow copper oxide, 15.4 tons; Fugrocide, 8.6 tons; red copper oxide and oil emulsion, 9.6 tons; nonsprayed check, 4.8 tons. Some varieties responded better to the sprays than did others. For example, yellow copper oxide on N. Y. State gave a 500 per cent increase in yield over that of the check plot, while, on Illinois Pride, the increase was only 69 per cent. The period of bearing was lengthened very greatly by spraying. Pritchard, normally an early variety, continued bearing throughout the season when sprayed with yellow copper oxide.

*Maintaining Quality of Tomatoes by Delayed Spraying.* JOHN W. HEUBERGER AND JAMES G. HORSFALL. Ten years of field research on fruit quality are summarized. When *Alternaria solani* and/or *Septoria lycopersici* attacked, sprays reduced defoliation resulting in deeper red color and less sunscald and growth cracks. Spraying made fruits markedly firmer in 1938. Puncture readings by R. F. Suit in 1939 showed that spraying increased firmness by 11 per cent. Spraying reduced blossom-end rot in 1934, 1938, and 1939. Spraying increased percentage of U. S. No. 1 fruits in 1939 from 51.4 for nonsprayed to 66.0 for Bordeaux, and 70.6 for yellow cuprous oxide. "Insoluble" copper materials, in general, gave no spray injury. Delaying the applications reduced it for Bordeaux. In 1938 and 1939, respectively, stem-end-rot lesions (*A. solani*) were reduced from 34.1 and 7.2 for nonsprayed fruits to 19.6 and 4.5 for Bordeaux, to 21.3 and 4.7 for red copper oxide, and to 26.1 and 5.6 for copper oxychloride "A." Percentage anthracnose (*C. phomoides*) was reduced in 1938 from 29.2 for nonsprayed to 15.9 for red copper oxide and 12.3 for Bordeaux, and in 1939 from 7.5 for nonsprayed to 1.8 for yellow copper oxide, and 0.9 for Bordeaux. Delayed spraying effectively and inexpensively maintained fruit quality at a high level, despite disease.

*Strains of the Fire-blight Organism.* E. M. HILDEBRAND. The cause of sporadic character of fire blight and the fluctuations in severity of its attacks has been sought in the pathogen. This study employed 136 isolates from diverse sources, involved numerous morphological, physiological and pathogenetical experiments and required 5 years for its completion in 1937. The most satisfactory suspect material for measuring grades of virulence was slices of green Kieffer pear fruits. The effect on virulence of successive bi-weekly transfer to nutrient broth demonstrated more variability than stability during 5 years. The smallest organisms were commonly most virulent. The mean length meas-

urements of 63 cultures ( $1.59 \mu$ ), when classified according to virulence, were: 9 high,  $1.42 \mu$ ; 12 medium,  $1.57 \mu$ ; 18 slight,  $1.61 \mu$ ; 18 nonvirulent,  $1.59 \mu$ . Differences in frequency of cells possessing flagella, number of flagella per cell, and length of flagella seemed not to be significant, except for slightly virulent or nonvirulent cultures. Physiological experiments employing 41 carbon and 18 nitrogen compounds and numerous miscellaneous cultural and other tests, failed to locate a more reliable criterion for evaluating strains of the organism than pathogenicity. While numerous small differences obtain between individual strains or isolates, no strain is believed of significance in any breeding program for disease resistance.

*Yellow-red or "X" Disease: A New Threat to Peach Industry.* E. M. HILDEBRAND AND D. H. PALMITER. Originally discovered in Connecticut in 1933, the Yellow-red or "X" disease is now found nearly across the Continent. All varieties of peach seem to be susceptible, but only the chokecherry (*Prunus virginiana*) among wild plants. Leaf symptoms appear on peach in mid-June and are continuously present thereafter till autumn. The sequence—leaf yellowing, irregular red spots, shot-hole, defoliation—is repeated year after year, but rarely results in death to orchard trees. However, in the greenhouse, trees die when completely defoliated. Fruit drop accompanies leaf fall, but small mummies often remain attached. On the chokecherry, the sequence—beautiful red and yellow foliage, coloring and rosetting and finally rosetting and death—usually appears in successive years, although with considerable overlapping. Maximum injury encountered in peach orchards during the first and second seasons was 62 and 96 per cent of trees infected. The extent of symptoms on individual trees ranged from single shoots to entire trees. Following inoculations by budding, symptoms may be delayed a year or longer. Eradication of the chokecherry, on which the disease spreads very rapidly, from the neighborhood of peach orchards is the best control measure. Chemicals offer most promise in preliminary experiments.

*The Response to Certain Vitamins of Fourteen Species or Strains of the Myriangiales.* ARTHUR B. HILLEGAS, FREDERICK KAVANAUGH AND ANNA E. JENKINS. The variations in the response of 14 species or strains of *Elsinoë*, *Myriangium*, and *Sphaeccloma* to different vitamins have been observed. The cultures were grown in a medium of mineral salts, asparagine, and dextrose, and the same medium to which different vitamins had been added. Eleven species or strains grew for 3 subcultures in the absence of thiamin. The 3 remaining species required some growth substances other than the water-soluble vitamins of known composition.

*Succession of Soil-inhabiting Fungi Attacking the Roots of Maize.* WEN-CHUN HO AND I. E. MELHUS. About 11,000 isolates have been made from roots of maize collected in various parts of Iowa since 1935. The fungi isolated may be divided into 3 groups based on disease-inducing capacity. (1) Severe: *Pythium graminicola*, *Rhizoctonia solani*; (2) moderate: *Diplodia zeae*, *Gibberella saubinetii*, *Pythium debaryanum*, *Helminthosporium sativum*, *Penicillium oxalicum*; (3) slight to none: *Trichoderma lignorum*, *Aspergillus niger*, *A. flavus*, *A. tamarii*, *Fusarium moniliforme*, *Basisporium gallarum*, *Phytophthora cactorum*, *Monotospora* sp., and *Fusarium* spp. The succession of these fungi on maize roots is influenced by soil and environmental conditions. In most cases *Pythium debaryanum* became parasitic before and following emergence causing seed decay and stunting. Later, *Pythium graminicola* appeared on seedling root tips, inducing rapid necrosis. Concurrently, *Rhizoctonia solani* often appeared on the mesocotyls, eventually causing decortication of the roots. Subsequently, such organisms as *Gibberella saubinetii*, *Helminthosporium sativum*, or *Penicillium oxalicum* produced further necrotic lesions on the mesocotyl and basal part of the roots. Still later, *Fusarium moniliforme*, *Fusarium* spp., *Gibberella saubinetii* (certain strains), *Trichoderma lignorum* and *Monotospora* sp. were observed in root lesions. On host maturity, *Diplodia zeae*, *Fusarium* spp., etc., may develop extensively in roots and crown, leaving no evidence of the earlier prevalent organisms.

*A Comparison of Pathogenic Races of Fusarium bilbigenum var. niveum.* DONALD E. HOFFMASTER. Wilt of watermelon, caused by *Fusarium bilbigenum* var. *niveum*, is prevalent in parts of West Virginia. A wilt of muskmelon in the same locality often is attributed to this fungus, but a survey has not revealed a *Fusarium* pathogenic to muskmelon. Inoculations with many different isolates of the watermelon pathogen have consistently failed to produce wilt on muskmelon. Cultures of the muskmelon wilt pathogen obtained from Minnesota have failed to wilt watermelon, while readily causing wilt of muskmelon. The two pathogenic races are culturally and morphologically alike. Sectors obtained from the two pathogenic races of the fungus showed wide variation in virulence but no change in host specificity. Sectors of several other wilt-producing *Fusaria* showed

similar variations in virulence, but no change in host preference. Pathogenic *Fusaria* probably originated from nonpathogenic soil *Fusaria* by dissociation; but no evidence was obtained to support the theory that the muskmelon pathogen originated from the water-melon pathogen in this manner.

*Relation of Color to Fungicidal Value of Insoluble Copper Compounds.* JAMES G. HORSFALL AND JOHN W. HEUBERGER. Previously, it was reported (*Phytopath.* 29: 303) that fungicidal value (*i.e.* spore-killing capacity) of cuprous oxide increased as the wave length of reflected light shortened. Two years' laboratory tests at Geneva, New York, showed that a similar color relationship occurred among the so-called insoluble basic cupric fungicide powders, both pure and commercial. As the wave length decreased from 5100 Å (green) for basic sulphate and one oxychloride through 5000 Å (greenish blue) for silicate, 4900 Å (blue green) for zeolite, 4800 Å blue for another oxychloride, to 4700 Å (bluish purple) for oxbordo, the fungicidal value (no data on field performance) increased. It seems noteworthy that, irrespective of anion, a single curve fitted all points for fungicidal value and wave length. Although fillers complicate the procedure, in the few cases where sound data could be obtained, it appeared that the fungicidal value relationship is one of particle size, as in cuprous oxide, *i.e.*, the shorter the wave length the smaller the particle. It seems significant that the cuprous oxide curve did not coincide with that for the cupric materials. This indicates that the cuprous oxides were more fungicidal than would be indicated by their wave lengths.

*Bleeding Canker of Maple.* F. L. HOWARD AND N. CAROSELLI. This disease is prevalent in several species of maple in home grounds, parkways, and nurseries in Rhode Island. Repeated subjection to Koch's postulates has proved the pathogen to be *Phytophthora cactorum* (identified by C. M. Tucker). The characteristic symptom is a reddish ooze from small fissures in cankers of the trunk and the scaffold branches. Infected inner bark, cambium, and sapwood develop a reddish brown necrotic lesion, which often exhibits an olive green margin. The fungus produces a toxic substance, which causes wilting of leaves and dieback of branches as secondary symptoms. Since the systemic nature of the infection indicates possible control by internal medication, a simple injection technique has been developed. A di-nitro-cresol compound has given promising results in laboratory and field trials, since it does not injure the healthy tissues of the tree and acts as an antitoxin and a pathogen-inhibiting agent.

*Peach-mosaic Virus Strain Studies.* LEE M. HUTCHINS AND L. C. COCHRAN. In commercial J. II. Hale peach orchards in southern California, in which all of the trees are affected with peach mosaic, some trees, known to have been affected for 5 years, have not developed the severe symptoms expected on the J. II. Hale variety. Orchard surveys tend to show a shock effect after initial infection, followed by partial recovery, but not sufficient to account for individual tree differences. When mildly affected trees are budded with buds from severely affected neighbor trees, they develop severe mosaic symptoms; severely affected trees, conversely treated, remain severely affected. Groups of 10 J. H. Hale peach nursery trees were budded from mosaic-affected orchard peach trees showing, respectively, mild, medium, and severe symptoms. Within each group, all inoculated trees developed almost identical symptoms, and the latter corresponded in most cases with the degree of severity of symptoms in the orchard-tree source. Certain severely mosaic-affected orchard peach trees produced apparently normal sportlike shoots. Among several shoots thus observed, some have later become severely affected; others, after 3 years, still appear normal. Buds from the nonmottled shoots, grafted in peach nursery trees, transmitted mild mosaic, whereas those from severely mottled shoots transmitted severe mosaic. Strains of the peach-mosaic virus appear to be demonstrated.

*A Bulb Disease of Lilies Caused by Fusarium spp.* E. P. IMLE. A heretofore unreported bulb rot of certain species in the genus *Lilium* particularly *L. candidum* and *L. testaceum*, has been under observation and study for the past 3 summers. Isolations from more than 100 different bulbs from widely separated parts of the United States and from Canada, Holland, and France have consistently yielded strains of *Fusarium*. Some cases of imported French candidum lilies showed from 20 to 36 per cent diseased bulbs in September, 1938. Imported stocks examined in 1939 revealed only 4 per cent diseased bulbs. Inoculation of wounded seedlings, bulblets, and larger bulbs have reproduced the disease symptoms but some evidence indicates that wounds are not necessary for infection. Symptoms may develop after the bulbs are in storage or transit. The fungus usually is confined to the base of the scales, severing them from the basal-plate portion of the bulb. Plant symptoms consist of a yellowing or purpling and premature dying of the basal leaves. Flowering stems, when produced at all, are stunted and inferior. Diseased bulbs, when not destroyed outright, tend to split up and become smaller. Partial control by disinfection of diseased dormant bulbs has been obtained by a 30 min. treatment with 1: 50 formaldehyde.

*Elsinoë and Sphaeloma Species in the United States, Puerto Rico, and Guam.* ANNA E. JENKINS AND A. A. BITANCOURT. A new species of *Elsinoë* on the rubiaceous host, *Randia*, from Puerto Rico, is described and the discovery and identification of *Sphaeloma batatas* on *Ipomoea batatas* from Guam (1937) is reported. Including these 2 species and *S. lippiae* and *Sphaeloma* on *Plantago rugelii* recently discovered in Indiana, 18 described species of *Elsinoë* or *Sphaeloma* are now known in the United States, Puerto Rico, and Guam. Their host plants represent 17 families ranging from the Juglandaceae to the Compositae. The number of *Elsinoë* or *Sphaeloma* species known in continental United States has more than quadrupled in the last 10 years, whereas the world list has increased at least tenfold, the species now numbering as many as a hundred. The largest number of new species from any one country has been discovered in Brazil. Of the 18 species now known to occur in the United States, Puerto Rico, and Guam, practically half had become established as early as 1869-1893, and 3 and possibly 4 between 1911 and 1926. Five others are without record before 1930. Representative specimens constitute Fascicle III of "Myriangiales Selecti Exsiccati," of which Fascicle I represents the species known in South America up to January 1936.

*Bacterial Leaf Diseases in Tobacco Beds in Relation to Field Infection.* E. M. JOHNSON AND W. D. VALLEAU. In a 1937-1939 survey in western Kentucky, of 533 beds receiving Bordeaux twice, only 2 had wildfire and 2 angular leaf spot. Infections appeared in these beds 3 days after treatment and probably were present when treated. In this area wildfire was present in 278 and angular leaf spot in 266 of 777 nontreated beds. Tobacco, set from infected beds, develops infection soon after setting and the diseases become destructive sooner than in fields from disease-free beds. In 1939, 47 fields set from beds, 29 of which had wildfire, 13 of which had both wildfire and angular leaf spot, and 13 of which had angular leaf spot, developed 2 to 6 weeks after setting, 1 to 100 per cent wildfire infection and 1 to 76 per cent angular-leaf-spot infection. Despite low rainfall in the last half of the growing season, the damage in fields from these beds was 1 to 50 per cent. No damage occurred in 100 fields set from Bordeaux-treated beds, and infection was rare. Bordeaux gave similar results in 1936-1937. In 1938, a rainy season, Bordeaux delayed injury until a few days before cutting time, and some satisfactory crops were harvested.

*A Buff Smut of Fall Panicum.* H. W. JOHNSON, H. A. RODENHISER AND C. L. LEFEBVRE. In an experiment made at Arlington, Virginia, in 1938, to determine the effect of incubation period temperature on the pathogenicity of *Sorosporium syntherismae* on *Panicum dichotomiflorum*, 70.9, 72.5, 53.3, and 22.2 per cent smutted plants developed following 26 days' incubation of chlamydospore-dusted seed at temperatures of 5, 10, 15, and 20° C., respectively. One smutted plant in this experiment was observed to have buff sori composed of hyaline, smooth-wall chlamydospores, while the other 307 smutted plants all had the common black sori composed of brown, echinulate-wall chlamydospores. Inoculations made in 1939 with chlamydospores of the buff-type smut resulted in 68 smutted plants, all of which produced only buff sori. Parallel inoculations with chlamydospores of the dark-type smut resulted in 240 smutted plants, all of which produced only black sori. Single chlamydospore cultures of the two types of smut were established and the optimum temperature for the growth of each type on potato-dextrose agar was approximately 20° C. The cultures of the buff smut were mycelial and made greater radial growth than did cultures of the black smut, which were of the sporidial type. It appears that the buff smut is a result of mutation in *Sorosporium syntherismae* and that the change may involve several genetic factors.

*Inoculation of Bean with Extracts from Other Healthy Legume Species.* JAMES JOHNSON. Juice extracted from 122 apparently healthy species (representing 50 genera) of the Leguminosae was rubbed with carborundum into leaves of young healthy beans (*Phaseolus vulgaris* L. var. Stringless Green Refugee). No species has consistently yielded a virus on the host plants used. Juice from single plants of one species, *Lathyrus tingitanus*, has been applied as inoculum more than 100 times to several bean plants and has induced systemic symptoms in every instance. Serial transmission, however, failed; the properties of the agent are not characteristic of a virus. The response of the bean to the extract from the Tangier pea (*L. tingitanus*) is tentatively interpreted as an allergic reaction. Two seemingly new viruses have been obtained from inoculations with apparently healthy plants. One of these has been obtained several times and the other only once in several attempts. It is logical to assume that these viruses were seed-borne and that the host species are symptomless carriers. If some part of the protoplasm of one species should, however, find favorable conditions for growth in the cells of another, when properly introduced (viroplasm theory), it is believed that consistent results should not, necessarily, be expected from such transfers.

*A Rare Virus Disease on Tobacco.* JAMES JOHNSON AND ROBERT W. FULTON. In 1938 a 6-acre field of tobacco near Edgerton, Wisconsin, showed about 30 per cent of the plants infected with an apparently new ring-spot virus. Similar symptoms had not been seen in extensive field disease surveys in previous years, nor were they noted in other fields in 1938, and did not appear in the original field in 1939. Of the several ring-spot viruses described, the new virus resembles most closely tomato ring spot, as described by Imle and Samson. Because the chlorotic rings are usually broader than those of other known ring-spots, the virus is tentatively given the common name "tobacco broad ring spot." The virus has been transmitted to some species outside the Solanaceae, including cucumber and sunflower. It apparently is not transmitted by *Myzus persicae*. The thermal death point after 10 minutes exposure is 52° C., the dilution end point is about 1 in 750, and the tolerance to aging *in vitro*, 36–48 hours at room temperature. Protective inoculation trials with ordinary tobacco ring spot showed no immunological reactions. Such rare occurrences of virus infection is of some interest in relation to the nature and origin of virus diseases.

*A Bud-transmissible Chlorosis of Prunus cerasus.* G. W. KEITT AND C. N. CLAYTON. A chlorotic disease of sour cherry, previously called "physiological yellow leaf," has been transmitted by budding. A typically diseased tree shows chlorosis and abscission of the older leaves, usually beginning about 3 weeks after petal-fall. Length of twig growth does not seem markedly affected, but the spur system becomes progressively reduced. The trees become unprofitable, producing sparse crops of large fruits free from necrosis or bumpiness. In 1938, diseased buds were inserted in 24 healthy Montmorency trees in 3 orchards. Twenty trees showed typical chlorosis in 1939; 4 were doubtful. Only 3 of the inserted buds produced shoots. Healthy buds were inserted in 8 diseased trees in 3 orchards. Four grew into shoots, all of which showed the characteristic chlorotic leaves and defoliation. Fourteen control trees, in one of which healthy buds were inserted, showed no symptoms of the disease. Microscopic and cultural examination of diseased tissues revealed no causal fungus or bacterium. Orchard records for 4 years show a steady increase in incidence of the disease. The evidence indicates the disease is caused by a virus. Its further investigation and inquiry into its possible relations to previously described viral diseases are in progress.

*Eradicant Fungicidal Treatments in Relation to Apple-Scab Control.* G. W. KEITT, C. N. CLAYTON, AND M. H. LANGFORD. A small Wealthy orchard at Madison, Wisconsin, was sprayed in October, 1938, with a copper-lime-arsenic mixture. A similar orchard nearby was not treated. Neither was sprayed in 1939. Counts indicated reduction in perithecia per unit area of overwintered leaf surface in the treated orchard, as compared with the nontreated, of 98 per cent; of current-season lesions per leaf, May 29, 99 per cent; June 26, 96; of lesions per fruit, Sept. 1, 98. Sixty-six per cent of the fruit of the nontreated orchard was scabbed, 4 per cent of the treated. The floor of a 6-acre McIntosh orchard at Sturgeon Bay was sprayed before bud-break with 1 per cent "Elgetol Extra," 450 gal. per acre. A small orchard 0.3 mile away served as control. Counts indicated that, through the critical period until 3 weeks after petal-fall, the Elgetol treatment reduced by about 0.9 the unusually severe epidemic. Seven lime-sulphur treatments in the orchard that received Elgetol and 8 in a similar one that did not gave, respectively, 1 and 32 per cent scabbed fruit at harvest. Reduced summer-spray programs were more effective in the orchard that received Elgetol than in others that did not.

*Variation in the Germination of Chlamydospores of Ustilago zea.* M. F. KERNKAMP AND M. A. PETTY. Germination of chlamydospores of *Ustilago zea* was studied on agar drops on cover slips on van Tieghem cells, the environment being held constant. Extreme variations from the classical 4-cell promycelium were observed in the 14 crosses and collections studied. Likewise, there were variations in the number of promycelial cells giving rise to hyphal branches or sporidia, the number of chlamydospores from which sporidia grew directly, the number of chlamydospores having promycelial cells on 2 sides, and the number of promycelial cells producing neither sporidia nor hyphal branches. The germination type varies with the cross or collection of smut. For example, the number of 4-cell promycelia varied from 11 per cent to 78 per cent in different crosses and collections. The differences between crosses and collections were much greater than differences within cross and collections, and one type of germination usually predominated in each cross or collection. As the environment in which the spores were germinated was held constant, differences probably are due to genetic differences between the crosses and collections.

*Physiologic Race Determination in Puccinia coronata avenae.* C. H. KINGSOLVER AND H. C. MURPHY. A study was made of the reaction of 21 oat varieties to 81 single-pustule isolates of *Puccinia coronata avenae* collected in 16 states in 1938. These varieties

were those used for physiologic-race determination by Murphy in the United States and by Straib in Germany. Using Murphy's 13 varieties 19 races could be identified; with Straib's 15 varieties 46 races; with the 21 varieties 54 races. Twenty-four isolates identified as race 1 (Murphy) could be divided into 18 races by their reaction on Straib's varieties. Similarly, 28 isolates identified as race 6 (Murphy) could be divided into 16 races. Twelve isolates giving the same reaction on Straib's varieties could be divided into two races by reaction on Murphy's varieties and these were races 1 (3 isolates) and 6 (9 isolates). Of the 19 races identified with Murphy's varieties, 13 had been previously described and 6 were new. Races 1 and 6 in almost equal proportions comprised over 50 per cent of the total number. Race 47, first identified by Waterhouse in Australia, was identified in 2 collections—its first report in the United States. Race 7, one of the most prevalent races in the past, was identified only twice. Cooperative investigation between U. S. Department of Agriculture and Iowa Agricultural Experiment Station.

*Diseases of Citrus.* L. J. KLOTZ. A hundred color photographs and brief descriptions of nutritional, virus, mechanical, and parasitic disorders.

*Studies on Phytophthora citrophthora.* L. J. KLOTZ. Optimum growth temperature is 26° C. Mature zoosporangia are produced in 4 to 5 hours on alfalfa-stem cultures in running, well-aerated water at 24° C. In both light and darkness, decrease in temperature from any point between 10 and 29° C. induced swarming; an increase did not. At 9, 15.5, and 19 °C. many zoospores (5 to 20 per low-power field) were motile after 10 hours; but none after 24 hours; at 12.5° C. many (5 to 10) were motile after 24 hours; below 7° motility was lost in a few seconds and at 24° C., usually within an hour. At 25° C. germ tubes developed to an average length of 450  $\mu$  after 24 hours in nonsterilized tap water, 550  $\mu$  in distilled water, and 816  $\mu$  in 1-to-1 citron albedo sap; they penetrated lemon rind to a depth of 600  $\mu$  in the same period. One minute at 44.4° C., two at 43.9° C., and five at 43.3° C. were lethal to both zoospores and mycelium. They survived 9 days' continuous freezing at -6.5° C., but were killed by 1 day at -20° C. and 1 day at -20.6° C. The fungus on alfalfa stems in hardware cloth buried in the field was not viable after 24 hours at the 1-inch depth, the temperature attaining 36.4° C. in shade and 43.9° C. in sun. After 12 days it was not recovered from the 2-inch level, but was cultured from the 3- and 6-inch depths, temperatures reaching 44.4° C., 40.0° C., and 32.2° C., respectively, at the 3 levels. In autoclaved soil, with 5 or 10 per cent moisture, it did not survive 20 hours at 40° C. or 70 hours at 35° C., but was recovered after 43 hours at 35° C. One part cuprion in 300,000 prevented germination; 1/4-1/100 Bordeaux protected lemon fruits.

*Rapid Seed-corn Drying Checks Seed Infection.* BENJAMIN KOEHLER. Corn ears of several widely grown hybrids were hand-picked in the field in 1937 and 1938, when the grain moisture was about 30 per cent. The ears of each hybrid were divided at random into 3 lots of 120 ears each to determine the effect of different rates of drying on internal seed infection. Chambers with temperature and humidity control were used in which the ear corn was dried to 12 per cent grain moisture. One lot was dried at 106° F., 32 per cent relative humidity, for 4 days; another lot at 70° F., 65 per cent relative humidity for 4 weeks; and the third lot at 70° F., and 86 per cent relative humidity for 3 months. Rapid drying avoided internal seed infection to a marked extent in these tests with selected ears of sound appearance. Total internal seed infection with the various fungi concerned was 5.1, 18.3, and 69.0 per cent, respectively, for the 3 different drying conditions. *Fusarium moniliforme*, *Gibberella zeae*, *Nigrospora* spp. and *Penicillium* spp. were all markedly increased by retarded drying. Rapid drying, as now practiced by many hybrid seed corn producers, seems of some value in controlling disease.

*Seed Treatment for the Control of Bacterial Bean Blight.* K. W. KREITLOW. Seed treatments for control of bacterial bean blight were tested in laboratory and field. Laboratory tests of diseased seed have given information on length of time seed can be treated without injury, as well as effectiveness of treatments in killing the disease organisms. On the basis of this information, 4 disinfectants have been used in treating several different lots of diseased seed prior to planting in the field. Six separate treatments and plantings were carried out at weekly intervals in the spring of 1939. The total average blight percentage in all checks for all plantings was 23.6 per cent, whereas the total average blight percentage of the 4 treatments for all plantings was less than 0.20. Yields of from 2 to 7 times that of the checks were obtained, and the quality of the beans was correspondingly higher in the treated plots. Periodic germination tests on treated bean seed kept in storage revealed no important decrease in germination during 7 months. The following solutions were effective in controlling bean-blight bacteria without serious injury due to the seed coats slipping: (1) 1: 500 mercury bichloride in di-ethyl ether. (2) 1: 20,000 brilliant green in 50 per cent ethyl alcohol plus 3 per cent

acetic acid. (3) 1: 500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid. (4) 1: 20,000 gentian violet in 50 per cent ethyl alcohol plus 3 per cent acetic acid.

*A Necrotic Virosis of Cabbage.* R. H. LARSON AND J. C. WALKER. A virosis of cabbage associated with but distinct from mosaic has been observed at Madison, Wisconsin. The symptoms resemble those of black ring in the paucity of mottle and absence of vein clearing, together with the preponderance of systemic appearance of small necrotic rings or spots. Black or purple, irregular, necrotic, slightly sunken lesions occur on stems. This virosis is distinct from black ring in that it develops best at 22 to 25 degrees C. and is completely masked at 18° to 19°. The physical properties also differ from those of black ring and mosaic. The inactivation point is 50°, duration *in vitro* 24 hours at 22°, and the dilution tolerance 1 to 500. It is transmitted readily by mechanical means and by the cabbage and peach aphids. It affects systemically all crucifers tested and also beet, chard, spinach, cucumber, zinnia, calendula, petunia, *Nicotiana glutinosa*, *N. rustica*, *N. langsdorffii* and *N. repanda*. Local lesions only are produced on inoculated leaves of tobacco. (University of Wisconsin and Division Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.)

*A New Phoma Disease of Perennial Delphinium.* THOMAS LASKARIS. A hitherto unrecognized disease caused by a species of *Phoma* has been frequently found in plantings of *Delphinium* in New York, New Jersey, and Connecticut. In the field the disease produces cankers, usually near the crown, causing one or more cracks in the stem, which may extend upward for a considerable distance. Pyrenidia of the fungus can be found in the blackened tissue adjacent to the edges of these cracks and also on irregular black or brown necrotic areas along the stem. Another characteristic symptom is the blackening of tissue at the base of each leaf petiole on which areas pyrenidia develop in abundance. On old stems reddish-brown masses of pyrenidia may appear near the top. The fungus has been isolated from the areas mentioned above and from crowns and roots of other naturally infected mature plants. Recently the fungus has been isolated from reddish-brown areas on leaves and petioles of young seedlings in the greenhouse. Inoculations on greenhouse plants have been successful and there is no doubt as to the pathogenicity of the fungus. The importance of this organism as a cause of crown rot of perennial delphinium is being investigated.

*Fungi Associated with Scolytus multistriatus in Regions where Ceratostomella ulmi Has not Been Found.* J. G. LEACH. A survey has been made of the fungi associated with the smaller European elm-bark beetle in southwestern West Virginia where the Dutch elm disease has not been found. The following fungi appear consistently associated with the insect, and they fruit abundantly in the brood galleries in a manner adapted to effective dissemination by emerging beetles: 1. a species of *Graphium* that forms coremia in the tunnels in a manner closely paralleling that of *Ceratostomella ulmi*; 2. a white *Penicillium* that sporulates in the egg galleries during larval development; 3. a species of *Fusarium* that forms microconidia in Cephalosporium-like heads; 4. one or more species of yeast. These fungi are believed to play an important rôle in the economy of the insect by creating a microenvironment favorable for larval development. The beetle is multiplying rapidly in West Virginia in trees killed by phloem necrosis, and conditions exist that will be conducive to rapid spread of the Dutch elm disease if and when *Ceratostomella ulmi* gains access to the region.

*The Reniform Nematode as a Root Parasite.* M. B. LINFORD, JULIETTE M. OLIVEIRA AND FRANCIS YAP. A nematode (description in press) that necessitates erection of a new genus of the Tylenchidae occurs on Oahu, Territory of Hawaii, as a mildly pathogenic parasite of roots. Over 60 known host species represent 29 families of plants. Injuries include minute cortical lesions and mild hypertrophy in the stele. Larvae of typical eelworm shape develop in the soil, without feeding, into mature nonfeeding males and infective young females, both resembling larvae in size and shape. Females penetrate intracellularly into the cortex of roots, coming to rest with the feeding stylet inserted into an endodermal cell. This cell and nearby pericycle parenchyma enlarge and become densely protoplasmic. The posterior part of the female, usually on the root surface, enlarges to become reniform, then eggs are laid in a gelatinous matrix, forming a mass that covers the reniform body and adheres to root and soil. In naturally infested soil, of a moisture content near the wilting percentage for plants, the reniform nematodes exceeded 50 per cent survival during 36 weeks. In soil air-dried 33 weeks, with moisture dropping to  $\frac{1}{2}$  of the wilting percentage, there was more than 5 per cent survival. This nematode appears less tolerant to freezing in soil than does the root-knot nematode.

*Hyperauxing of Nodules of Red Kidney Bean, Soybean, and Garden Pea.* G. K. K. LINK AND VIRGINIA EGGERS. Using the *Avenda* coleoptile test and peroxide-free ether ex-

tracts of the test material, it was shown that the auxin content of nodules (1) of kidney bean is 3-10 times greater; (2) of soybean is 1.5 times greater; and (3) of garden pea is 2.5 times greater, respectively, than that of the roots that bore them. The auxin contents of nodule-free root systems of these plants, when grown in sterile quartz with Shive's complete nutrient solution, were approximately the same as those of nodule-bearing plants grown in garden soil.

*Dissemination of the New York Aster-yellows Virus and Its Leaf-hopper Vector, Macrosteles divisus in Endive Beds.* M. B. LINN. A study of the dissemination of the New York aster-, lettuce-, and endive-yellows virus has shown that the amount of yellows in certain endive beds is often considerable in plant rows immediately adjacent to bordering weeds, gradually decreasing in amount towards the far sides or ends of the beds. Yellows-distribution curves, having as coordinates percentage of infection and distance in feet from sampling point to weed borders, are typically logarithmic. If the logarithm of the percentage of infection be plotted against the distance in feet, the resulting curves are straight lines. This suggests that vector and virus dispersion, as measured by the incidence of yellows, may be expressed by constants that vary in value from one bed to another, and are conditioned by any factors influencing feeding and dispersion of the vector. Knowing the intercepts or points where the log curves intercept the coordinates, one may then postulate the highest percentage of infection that could be anticipated in the bordering weeds, and the distance in feet from the weeds to that point in the endive beds, where the former were no longer factors in the distribution of yellows in these beds.

*The Epidemiology and Control of Hop Downy Mildew.* ROBERT O. MAGIE. Under New York conditions, oospores of *Pseudoperonospora humuli* in the soil are the principal means of overwintering. Sporangia on detached leaves withstood drying for 27 days over calcium chloride or in the greenhouse at summer temperatures. Plants were sprayed with sporangial suspensions, placed in moist chambers at 10, 13, 18 and 24° C., then removed, and quickly dried after various time periods. Minimal period of wetting permitting infection at the two higher temperatures was 1½ hours and maximal infection occurred at 13° and 18° after 24-hour wetting periods. Removal of diseased shoots or application of calcium cyanamide to crowns retarded secondary infection, but did not permit a reduction in spraying. Bordeaux and cuprous oxide were more effective than other fungicides tested. Bordeaux injured young leaves and stunted cones. The addition of ¼ per cent cottonseed oil reduced these injuries. Bordeaux was outstanding in that rain drippings from sprayed leaves protected nonsprayed growth. Four biweekly applications, beginning the middle of June, of either Bordeaux 4-2-100 or cuprous oxide 1-100, with the oil, were very effective if thoroughly applied. It is suggested that spraying may be delayed until infection reaches the foliage 5 feet above ground.

*A Comparison of Methods of Laboratory Spraying for the Testing of Protective Fungicides.* S. E. A. MCCALLAN AND FRANK WILCOXON. The errors of testing fungicides in the laboratory arise from 2 major sources: biological, involving the fungus, and mechanical, arising from application of the spray. A comparison was made of 3 basic principles of applying sprays: freehand spraying, spray chambers, and settling towers. Uniformity and reproducibility of spray deposit were determined by spraying with a dye solution and measuring in a colorimeter. The coefficient of variation for spray deposits on replicate slides sprayed at (a) the same time, and (b) different times or days, was as follows: (1) freehand spraying with bulb atomizer, (a) 31.0 per cent, and (b) 98.7 per cent; (2) freehand spraying with controlled pressure and time - 8.0 and 63.0 per cent; (3) spray chamber, fixed nozzle, controlled pressure, concentration varied by time of spraying - 5.9 and 30.1 per cent; and (4) settling tower, controlled pressure and time, concentration varied by successive exposures - 4.5 and 12.8 per cent. Method (1) is entirely unsatisfactory. The chi-square test applied to toxicity curves for 2 fungicides on the spores of 4 different fungi gave the following mean chi-square/n values: method (2) 7.4, (3) 4.3, and (4) 2.6. A permanent stainless steel settling tower suitable for laboratory testing of sprays and dusts has been designed and constructed.

*Field Studies on Paradichlorobenzene in the Control of Tobacco Downy Mildew.* RUTH MCLEAN AND J. A. PINCKARD. Previous investigations have demonstrated the fungicidal value of paradichlorobenzene vapor to lie in its specific or eradicant action on the parasite without causing significant injury to the host. This important property of the fungicide allowed delayed seed-bed application of the vapor until infection had occurred and the sporangia of downy mildew appeared on seedlings. Following appearance of the parasite, in a given seed bed of 100 sq. yd., 3 to 4 lb. of No. 6 size crystals were broadcast on the ordinary cotton cover at 6 p.m. A cotton fumigation cover having 64 warp and 64 woof per inch was drawn over the treated seed bed, where it remained 12 hours. On farm-oper-

ated seed beds, 3 nightly treatments of 12 hours each suffice to eradicate the parasite when temperature was above 6 to 8° C. Single 12-hour treatments may suffice on warm nights, using wet or sealed fumigation covers. Treatments made nightly and on alternate nights with half the above amount of paradichlorobenzene were effective. Damage to seedlings resulted from the vapor at temperatures encountered during attempted day treatments of shorter duration. Vapor concentrations in seed beds were determined quantitatively and correlated with fungicidal effectiveness.

*Adaptive Parasitism of Phytophthora infestans.* W. R. MILLS. The virulence of *Phytophthora infestans* has been increased by passage through a series of potato plants representing 3 different levels of resistance. Varieties entirely immune from the original cultures become completely susceptible to the virulent ones. Plants representing a fourth level of resistance are immune from all cultures. Increase of virulence occurs while the mycelium is growing within the tissue of the resistant plant but not when the fungus is propagated in susceptible hosts. Somatic fragments (swarmspores) of increased virulence are selected by applying them to foliage of greater and greater resistance. The virulence of the identical cultures used in the experiments with potato has been progressively increased for tomato by 7 successive passages through foliage of that host, at which time the fungus produced a blight of tomato fully as severe as that caused by a culture taken from a naturally infected potato. Increase of virulence on tomato did not affect in any manner the virulence for potato. The converse also proved to be true. Once increased, virulence of cultures could not be reduced by long periods of growth on tubers and foliage of low resistance.

*Auxin Production by Ustilago zeae Grown on a Medium Free of Tryptophane.* J. E. MOULTON AND G. K. K. LINK. *Ustilago zeae* was grown on Ranker's synthetic nutrient solution in which dextrose is the source of carbon and the only organic substance present. The organism was grown on this medium for 8 weeks. Ether extracts were made from the fungus mat and the supporting medium, using the extractor and methods employed by Link, Wilcox, and Eggers (*Phytopathology* 28: 15, 1938). The extracts were applied to *Avena* coleoptiles and were found high in auxins. These results indicate that *Ustilago zeae* is capable of producing auxins when grown in a synthetic medium devoid of peptone or other sources of tryptophane. Work is under way on the relationship of auxins and gall production by strains of *U. zeae*.

*Effect of the 1938 Crown-rust Epiphytotic on Yield, Bushel Weight, and Lodging of Oats in Iowa.* H. C. MURPHY, L. C. BURNETT AND C. H. KINGSOLVER. A statistical study was made of the effect of the severe 1938 crown-rust epiphytotic on the yield, bushel weight, and lodging of 442 varieties and selections of oats grown in replicated yield tests at Ames and Kanawha, Iowa. Coefficient of infection (a product of percentage and type of infection) was more highly negatively correlated with yield and bushel weight than either percentage or type of infection. The 442 selections, mostly of hybrid origin and varying in reaction to crown rust from near immunity to complete susceptibility, were grouped according to their coefficients of infection. Their average yields ranged from 24.9 bushels per acre for those with 91-100 coefficient to 79.7 for those with 0 rust. The correlation between yield and coefficient of crown-rust infection was -0.8. For each unit increase in coefficient of rust, yield was decreased 0.31 bushels per acre at Ames and 0.27 at Kanawha. Bushel weight showed a similar high negative correlation with rust infection. Lodging was increased by increase either in yield or crown-rust infection, or both; and, since yield and rust were negatively correlated, the effect of lodging (caused by rust) on yield tended to be nullified. (Cooperative investigation between U. S. Department of Agriculture and Iowa Agricultural Experiment Station.)

*Progress in Onion-smut Control by Seed Treatment.* A. G. NEWHALL. Although the liquid-formaldehyde-drip method of controlling onion smut on muckland is usually effective in reducing the disease to less than 5 per cent, weight of the solution adds much to the labor of sowing. In the past 5 years formaldehyde dusts, used in a similar manner, have proved neither so effective nor so economical in money or labor. More promising results have been secured recently by employing organic fungicidal dusts in seed treatment. Slow, gradual release of the fungicide is considered an important element in their success. The first to be tested, Brassicol or Brassisan (20 per cent pentachlor-nitro-benzene) was too toxic to onion seedlings, although it controlled smut fairly well. Two other organic compounds have given good smut control with sufficiently unimportant seed injury to warrant field trials on a larger scale. Mean percentages of smut on seedlings in 3 fields in 1939 were checks 32 per cent, liquid formaldehyde 3.9 per cent, DuBay dust 120U (50 per cent tetramethylthiuram disulfide) 2.49 per cent, DuBay dust 1242CC (50 per cent ferric dimethyldithiocarbamate) 2.51 per cent. Dust needed per pound of seed (1: 1) requires a sticker, such as soluble cottonseed oil or rosin-lime-sulphur for best results.

*Onion Bloat or Eelworm Rot Caused by the Nematode *Ditylenchus dipsaci*.* A. G. NEWHALL AND B. G. CHITWOOD. A second outbreak of the bulb or stem nematode was discovered on the mucklands of New York State in the summer of 1939. The fact that this disease is not recorded for any other State is very likely due to failure to recognize it. General field symptoms may be taken for lightning injury, but their recurrence in the same location is an important difference. Seedling symptoms include a twisting of the cotyledon on emergence, a white discoloration, enlarged areas and later a broken epidermis on the first leaf. Symptoms on half-grown onions raised from sets include stunting, prostrate outer leaves, die-back symptoms above ground, and, late in the season, a softening of the outer scales. An increased number of splits and doubles often occurs, as well as many secondary rots. On a mature bulb the diagnostic symptom is a soft white mealiness of the inner surface of the outermost scale or two, in which location the nematode usually can be found in abundance. The pathogen also is often present in stem and leaf tissue of growing onions late in August. Infested bulbs are not characterized by excess moisture or disagreeable odor. Parenchyma continues to break down in storage. The puffy soft condition of the second scale has suggested the term "bloat."

*Studies on the Fungicidal Properties of Silver.* L. W. NIELSEN. Freshly precipitated silver arsenate, arsenite, carbonate, chloride, chromate, cyanide, dichromate, phosphate, nitrate, sulphate, colloidal silver, and 3 silver-soap mixtures were found to be strongly fungicidal to *Alternaria solani* when tested on excised potato leaves. Silver bromide, ferrocyanide, iodide, sulphide, thiocyanate, thiosulphate, and nucleinate were only weakly fungicidal. Under greenhouse conditions silver-lauryl sulphate and -oleyl sulphate precipitates and precipitated silver oxide with silver concentrations of 12.5 p.p.m. controlled late blight infection on potted potato plants, as well as 4-4-50 Bordeaux mixture. During the past summer silver-lauryl sulphate and -oleyl sulphate precipitates, silver dichromate, arsenate, cyanide, oxide, chloride, and colloidal silver were tested under field conditions. The protection given potatoes against late blight by silver chloride and silver-lauryl sulphate precipitate was inferior to that given by Bordeaux mixture, as was apple scab control with silver oxide, dichromate, and silver-lauryl sulphate precipitate. Laboratory studies have shown that the silver sprays tested adhere very poorly. Various organic and inorganic materials are being added to or combined with silver in an effort to increase its adhesiveness and still retain its toxicity. Sprays containing silver and sodium lauryl sulphate or sodium oleyl sulphate have injured in some cases.

*The Character of Supplements and Their Effect on the Performance of Copper Fungicides.* A. A. NIKITIN. An attempt was made to correlate the effect of supplements on the solubility and degree of adherence of copper fungicides. These studies were relative to the performance of copper fungicides when used in combination with supplements, both in the laboratory and in the field. The results secured on the study of the physical, chemical and toxic properties of copper fungicides definitely showed that the addition of supplements may greatly change the performance of copper fungicides. The prime function of supplements is to improve the physical properties of the copper fungicides, such as adherence and spreading. However, in performing this function, they should be chemically unreactive with the copper fungicides. It is essential that the supplements should be of such a character that they do not readily undergo oxidation, reduction or hydrolysis, on exposure to atmospheric action. In this respect, they should not change the solubility of the copper fungicide. It has been found that among the materials commonly available on the farm soya flour, wheat flour, casein, and corn oil make suitable supplements. Skimmed sweet milk improves the adherence and spreading properties of copper fungicides, and, in addition, insures a very good finishing of the fruit.

*Eradicant Treatments as an Aid in the Control of Apple Scab.* D. H. PALMITER. The value of eradicant treatments in reducing the primary inoculum of *Venturia inaequalis* was tested in 2 isolated McIntosh orchards on the same farm. One block of 55 trees received a thorough ground application of Elgetol ( $\frac{1}{2}$  per cent). Most of the second orchard received an application of a nitrate-arsenite solution ( $\text{NaNO}_3$ , 100 lb.,  $\text{CaAsO}_3$ , 4 lb., water 100 gallons), but one end was left untreated. These materials were applied at the green-tip stage with a spray gun and sufficient pressure to kick up the clumps of old leaves and force the chemicals to the lower layers. The ground was sprayed from 2 directions with a total of 500 gal. per acre. Scabbed fruit on nonsprayed trees of the 2 treated plots and the nontreated plot was 2.7, 3.4, and 72.0 per cent, respectively. Trees receiving only the calyx and 10-day sprays (flotation sulphur paste 6-100) had 2.0, 3.1 and 43.1 per cent infection, respectively; while those receiving 4 sprays had 0.2, 0.8 and 1.7 per cent infection, respectively. From these and previous years' data it is concluded that such eradicant treatments can reduce a potentially heavy ascospore inoculum to such low levels that mild fungicides may be used with comparative safety.

*The Soil Rot, "Pox or Pit," of Sweet Potato.* L. H. PERSON. Two types of symptoms have been recognized in Louisiana as an accompaniment of the disease or disease complex of sweet potatoes known as soil rot, pox, or pit. These are: (1) the symptoms first given by Halsted and characteristic of a widely distributed disease; (2) the symptoms of the very serious disease that has been spreading in Louisiana during the past 5 years. From potatoes affected with the latter, or the Louisiana disease, there has been isolated an *Actinomyces* capable of reproducing the disease. This organism is apparently new and will be described as *Actinomyces ipomoea*, n. sp. The place in the complex of those potatoes showing the symptoms first described by Halsted is not yet clear.

*A Laboratory Method for Determining the Fungicidal Value of Vapors and its Application to Paradichlorobenzene in the Control of Tobacco Downy Mildew.* J. A. PINCKARD AND RUTH MCLEAN. Atmospheric concentrations of paradichlorobenzene vapor, fungicidal to downy mildew infected tobacco seedlings growing in jars, were studied by a simple semi-automatic, air-saturation method. Known concentrations of the fungicide, approximating a precision of 3 parts per 1,000, were delivered to test plants continuously or intermittently as required. The sporangial cycle of the parasite being 6 days, vapor treatments of infected seedlings were not begun until the 3rd day after inoculation. Harmless prevention of sporangial formation was correlated with vapor concentrations for single 12 hour treatments, and for alternate 12-hour treatments. With repeated alternate treatments, concentrations as low as saturation at 0° C. (0.01 volume per cent) were fungicidal. Single 12-hour treatments were fungicidal if concentrations equal to saturation above 7° C. (0.02 volume per cent) were applied. Concentrations at 13° C., or above, (0.04 volume per cent) were injurious to plants after 12 hours. Paradichlorobenzene vapor causes changes in parasite or host, or both, resulting in destruction, or prevention, of parasitism without causing significant harm to the host. To be fungicidal the vapor must be soluble in the cell plasma. Since these solutions obey the gas laws approximately, atmospheric concentrations are, at best, merely indicative of the important tissue concentrations.

*Phytophthora Disease of Maples.* P. P. PIRONE. During 1938 and 1939 hundreds of maples, especially *Acer platanoides*, have died throughout New Jersey. An early symptom of the disease is a thin crown resulting from a decrease in the number and size of the leaves. Trees die within a year or two following this period of weak vegetative growth. The presence of cankers at the base of the trunk near the soil line is the most striking symptom. The inner bark, cambium, and often the sapwood are reddish brown in the cankered area. Death occurs when the cankers completely girdle. Isolations from cankered and discolored areas yielded a fungus identified by C. M. Tucker, University of Missouri, as belonging to the *Phytophthora cambivora* group. Pathogenicity of this fungus has been established; it produced typical symptoms on at least 12 artificially inoculated trees; in all cases it was successfully reisolated. Infection resulted only when trees were wounded just before the inoculum was applied; no infection occurred when the fungus was applied to unwounded maple bark. Wound inoculations of young rhododendrons resulted in symptoms typical of the disease induced on it by *P. cambivora*. Application of the *Phytophthora* in wounds on *Cornus florida* did not produce infection.

*Brown Scale Disease of Easter Lily.* A. G. PLAKIDAS. During the last few years the brown-scale disease, a serious trouble of the Easter lily, also known locally as "brown bulb" or "black bulb," has become increasingly worse in the lily-growing district of Louisiana. The disease—apparently soil-borne—is characterized by a brown discoloration, mainly of the outer scales, although smaller brown spots may be scattered locally on the inner scales. When the bulbs are first dug, the brown areas are more or less superficial, the injury to the tissue rarely extending deeper than about  $\frac{1}{2}$  to  $\frac{1}{4}$  mm. Later, in storage, the affected scales shrivel, dry up, and turn dark brown to black and the bulbs become unmarketable. Repeated platings from affected tissue yielded mainly 3 fungi, *Penicillium* sp. (probably *P. cyclopium*), *Fusarium* spp., and one which has been tentatively identified as *Vermicularia* sp. Typical symptoms have been produced on healthy bulbs planted in sterilized soil inoculated with the last named fungus, either alone or in combination with the *Penicillium* or the *Fusarium*, or both. As neither the *Fusarium* nor the *Penicillium*, alone or together, produced infection, the *Vermicularia* appears to be the cause of the brown-scale disease.

*Effect of Some Mineral Nutrients on Development of Clubroot (*Plasmodiophora brassicae*).* DEAN E. PRYOR. Several resistant and susceptible crucifer varieties were grown in sand artificially infested with *Plasmodiophora brassicae*. Sulphur, nitrogen, and potassium were varied in different experiments so as to produce: (a) plants showing deficiency symptoms, (b) plants growing normally, and (c) plants having pronounced vegetative vigor resulting from an extra supply of nitrogen or potassium. In comparison with the "complete" solution, the number of club-bearing susceptible plants was, in

general, increased slightly by an abundance of potassium, more by an abundance of nitrogen, and most by the absence of sulphur or nitrogen; it was decreased markedly by insufficient potassium. The number of resistant club-bearing plants was increased somewhat by high nitrogen, more by sulphur or nitrogen deficiency, and decreased by insufficient potassium. An abundance of potassium was not conclusive in its effect. On some otherwise healthy plants, a small gall, 1 or 2 mm. in diameter, appeared usually but not always at the base of a branch root. Fewer susceptible plants deficient in sulphur or nitrogen had galls. The number of resistant plants with galls was increased by the absence of sulphur or by high nitrogen and was decreased by high or low potassium. The behavior of the other nutrition series with respect to these overgrowths was not conclusive.

*Control of Seed- and Soil-borne Diseases of the Potato.* CHARLES S. REDDY AND G. N. DAVIS. Five potato varieties were used in an experiment to reduce virus diseases in potato seed lots to a minimum by repeated greenhouse indexing and increasing in isolated plots. These stocks were indexed each spring and grown on mineral soils in North Central Iowa. The percentage of virus-infected tubers decreased annually for 4 consecutive years (60 to 24 per cent), but increased the fifth to 45 per cent. The percentages of infected tubers for the 5-year period were 59.3, 31.5, 28.7, 24.0, and 44.7. No new seed stock was added during this period. However, 2 virus diseases, witches'-broom and calico mosaic, not present in the original stock, appeared the third year. It was concluded that, under Iowa conditions, the production of virus-free seed potatoes by greenhouse indexing is impractical. Efforts to find a better potato seed-piece treatment to control scab and Rhizoctonia have given data on yields when these diseases are largely controlled and on the fungicidal efficiency of different chemicals. It was found that the time requirement for treatment was influenced by surface tension of the liquid and that duration of treatment could be controlled by double dips, the second dip changing the nature of the fungicide. Data were obtained on the use of dust fungicides as potato-seed-stock treatments.

*White Pine Selected in Blister-rust Areas.* A. J. RIKER AND T. F. KOUBA. The invasion of unprotected Wisconsin areas by *Cronartium ribicola* has caused the death of most small white pine trees. Perhaps 1 out of 300 to 500 trees, however, survived several years in the midst of abundant natural inoculum and was apparently free from blister-rust infection. This circumstance suggested a search for young cone-bearing trees, without evidence of disease, in areas where rust had been severe for years. A number were found, and 163 such trees in four Wisconsin areas were selected during the falls of 1938 and 1939. These trees had survived natural inoculation for 15 to 20 years without any apparent cankers. Recent surveys showed approximately 10,000 to 60,000 feet of *Ribes* live stem per acre. It seems reasonable that some of these trees may be rust-resistant. Cones were collected in 1938 and 1939, and the seed from each tree was planted in separate rows in the nursery. Likewise, veneer grafts with scions from 40 parent trees were successfully made in the spring of 1939 and are now growing. When the seedling trees are old enough, it is planned to subject them and also grafts from the parents to artificial, as well as natural, inoculation.

*Studies on Environmental Factors Affecting Infection and the Development of Bunt in Wheat.* H. A. RODENHISER AND J. W. TAYLOR. Differences in percentages of bunt infection were obtained when wheat seedlings were grown in Hempsted silt loam from St. Paul, Minn., and in Mendon loam from Logan, Utah. Differences in effect were contingent, however, on incubation-period temperature, the effect of soil type being marked at 5° C. and not at 10° and 15°. Steam sterilization of Mendon loam effected uniform reduction in percentages of infection in Marquis wheat at each of these 3 incubation temperatures. Steam sterilization of Hempsted silt loam caused marked effect at 2 of the 3 incubation temperatures. At 5° it increased infection 63.7 per cent; at 10° there was no significant difference; and at 15° it decreased infection 58 per cent. Increases in infection were obtained with diseases in soil acidity from pH 4.8 to a point approaching neutrality. The most marked effect on infection was from pH 4.8 to 5.5. Increased bunt was obtained in Hope and Marquis with increased day length. Hope plants, exposed to light for 24, 10-11, and 8 hours daily, developed 64.1, 17.5, and 0.8 per cent of smut, respectively. The corresponding percentages for Marquis were 32.7, 17.8, and 1.9.

*Properties and Purification of Alfalfa-mosaic Virus.* A. FRANK ROSS AND W. M. STANLEY. Tobacco plants inoculated with alfalfa-mosaic virus show a rapid increase in the virus activity of their extracted juices until about the 10th day following inoculation. Subsequently, they show a sharp decline until the activity is less than one-tenth of the maximum reached. The virus is partially inactivated by freezing whole plants in a cold room held at -14° C. and to a lesser extent by rapid freezing with solid carbon dioxide. The extracted juice may be frozen by the latter method without markedly affecting virus

activity. The virus is inactivated less rapidly at 4° C. than at room temperature and apparently is unaffected by certain reducing systems. It is most stable between pH 6 and 7. When juice from diseased plants is centrifuged for 1½ hours at 4° C. in a field of approximately 60,000 times gravity, the supernatant liquid is essentially free of virus and most of the activity can be recovered by dissolving the pellets. Purified preparations containing protein, phosphorus, and carbohydrate have been obtained by means of differential centrifugation.

*Physiological Specialization in Cercospora oryzae.* T. C. RYKER. Cercospora leaf spot is the most serious disease of Blue Rose rice, the variety normally comprising about 75 per cent of the Louisiana acreage. Resistant varieties have appeared to be the most logical means of control. Several disease-free plants were collected in a heavily-diseased field of Blue Rose in 1936. From these a selection, 2854-3, was obtained that was resistant to *Cercospora* and at the same time indistinguishable from Blue Rose in type. However, in certain artificial inoculations made in 1939, this variety was completely susceptible, suggesting the possibility of more than one pathogenic race of the fungus. To test this, 4 varieties, Blue Rose, Fortuna, 2854-3, and Caloro were inoculated with 20 cultures of the fungus. At least three distinct groups were identified: group 1, to which Blue Rose was susceptible and the other 3 varieties resistant; group 2, to which 2854-3 was susceptible, Blue Rose moderately susceptible, and Caloro and Fortuna resistant; group 3, to which Blue Rose and Caloro were susceptible and 2854-3 and Fortuna resistant. Field observation suggests the occurrence of still additional forms. However, certain of the resistant varieties appear to be resistant to all strains of the fungus.

*Seed Transmission of Tomato Mosaic.* R. W. SAMSON. Commercial importance of seed transmission of tomato mosaic was suspected when 170 acres, contracted by one Indiana canning company for the production of tomato pulp and seed, was found to be 100 per cent infected with this disease. The acreage had been set with transplants grown from seed of mosaic-infected plants. The transplants were started in a greenhouse, transplanted to coldframes, and subsequently transplanted to the fields. Mosaic virus on seed saved from this acreage was demonstrated by rubbing water extracts of numerous samples onto leaves of Early Golden Cluster bean and Jimson weed. Brown, necrotic spots, identical with spots produced with diluted juice of tomato plants infected with mosaic virus, collected from plants in the canning acreage in question, developed on the rubbed leaves. No mosaic symptoms appeared on 10,000 seedlings grown to the 5th leaf stage from this seed. Seed transmission apparently did not occur. Several hundred acres, set with transplants from mosaic-infested seed from other sources and grown in greenhouses and coldframes, were found to have a high percentage of mosaic. Elimination of one or both of the usual transplanting operations appears to control the tomato mosaic commonly occurring in Indiana, as shown by the fact that several thousand acres of canning tomatoes, set with field-grown transplants or seeded directly, were found virtually mosaic-free, even though the seed came from mosaic-diseased plants.

*Cultural Variation and Physiologic Specialization of Actinomyces scabies.* LAWRENCE A. SCHAALE<sup>1</sup>. Isolations of *Actinomyces scabies* were made from potato tubers from different geographic areas. Several cultural types often were obtained from individual pustules. Comparative studies of a large number of isolates grown on several nutrient media proved the existence of cultural races that differ in rate and type of growth, zonation, amount and color of pigmentation in the media, and in their tendency to sector. By inoculating differential varieties of potatoes many distinct parasitic races were recognized. *Actinomyces scabies* is unstable. Monosporous lines from certain cultures produced numerous sectors on potato-dextrose agar. Some of these variants continued to sector, yielding a variety of distinctly different cultural types. No line was completely stable, although a few lines produced only a single variant. It is possible that new parasitic races may arise from variants produced either in the soil or on the tubers. The existence of numerous parasitic races and the production of variants should be taken into consideration in breeding for resistance to scab.

*Growth Stimulation of Diplodia zeae.* G. SEMENIUK. On Czapek-Dox liquid medium, plain agar, and glucose agar, *Diplodia zeae* makes slow growth, while with additions of small amounts of water extracts of organic materials such as potato, carrot, cornmeal, oatmeal and pith of mature corn stalks, marked increases in growth rate follows. Stimulating properties were found in the aqueous solution of spore suspensions used for seeding purposes, which were obtained from cultures growing on whole-oat-extract agar. Such stimulating properties were not possessed by the liquid filtrates of

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31- or 36-day-old *D. zeae* cultures initially containing organic extracts or different nitrogen sources. Vitamin B<sub>1</sub> in amounts from 0.01 to 50 gamma per 25 cc. of liquid medium, had no effect, while white and yellow cornmeal were equally effective. The addition of these stimulating water extracts to the solid media increased pycnidial formation. Mycelial yields on Czapek-Dox medium with different inorganic nitrogen sources were greater in the presence of carrot decoction than in its absence. No marked effect was observed with bacto-peptone. The greatest stimulation occurred with thiourea, NaNO<sub>2</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub>. The NH<sub>4</sub> radical appears to be a readier source of nitrogen than the NO<sub>3</sub> radical.

*Seedling Infection of Maize by Diplodia zeae in Steamed and Nonsteamed Soil.* G. SEMENIUK. Equal amounts of *Diplodia zeae* inoculum were introduced into steamed and nonsteamed soil. The severity of corn seedling infection produced was from 10 to 50 per cent lower in the nonsteamed soil. Similar marked differences were obtained with field soil and compost, whereas only small differences existed between steamed and nonsteamed sand. Naturally infected seed was as severely diseased in nonsteamed as in steamed soil. In steamed soil containing inoculum, death of seedlings occurred at temperature intervals of 16-18, 20, 24-26, and 29-30° C. when the plants had attained a height of 2.0-6.0 cm. In nonsteamed soil containing inoculum, the seedlings approached the height of plants grown in soil without inoculum, even though the mesocotyl and roots were severely diseased. No marked temperature effects could be detected on seedling infection in nonsteamed soil. Maintaining seed in contact with inoculum in moist compost for 0, 2, 4, 6, and 10 days at 13.3° C. previous to transferring to a more favorable temperature for growth (20° C.) did not result in any greater infection in the nonsteamed soil. Delayed planting resulted in healthier seedlings. The antibiotic activity of other soil micro-organisms towards *D. zeae* may be the cause of the differences in the severity of infection observed.

*The Prevalence and Destructiveness of Plant Diseases in Iowa from 1850 to 1937.* D. R. SHEPHERD. In a study of records (dating back to 1858) of plant diseases in their relation to Iowa agriculture, 280, affecting 34 crops, were considered. Serious early losses from potato rot, thought to be late blight, were reported in 1858, 1865, 1866, 1869, 1876, 1885 and 1886. Fire blight of pear and apple became destructive in 1852 and did untold damage during the following 40 years. Consequently, much attention was focused on this and other orchard diseases. Early destructive outbreaks of rust of wheat (stem rust-leaf rust) were reported for 1859, 1876, 1878, 1880, 1890, 1893. Wheat scab caused widespread loss in 1865. Losses have resulted in the partial to complete elimination of some crops and a shift in acreage to those less vulnerable to attack. Stem rust, leaf rust, and scab appear to have been important contributing factors in the elimination of spring wheat as an Iowa crop; fire blight has been largely responsible for the almost complete elimination of pear culture; potato diseases in general have made potato culture in Iowa unprofitable; cabbage yellows ruined a thriving cabbage industry; and on Muscatine Island sand lands, watermelon wilt left only the remnant of a profitable watermelon industry in its wake.

*Effect of Nitrogen Nutrition on Virus Multiplication in Tobacco.* ERNEST L. SPENCER. The correlation previously reported between high-nitrogen nutrition and high virus concentration in Turkish tobacco plants (*Nicotiana tabacum*) has been found to be due to an increase in the rate of virus multiplication. Plants grown in nutrient sand cultures, supplied with a complete nutrient solution containing either 20 or 200 mg. of nitrogen per 100 cc., were inoculated with tobacco-mosaic virus when in the 3- or 4-leaf stage. At intervals, the juices of representative plants were assayed for virus concentration. On the 5th day after inoculation, little difference could be detected in the virus concentration of juices from the two sets of plants, but on successive days thereafter the virus concentration in juice from plants receiving 200 mg. of nitrogen became progressively higher than that in juice from plants receiving only 20 mg. of nitrogen. Since the two sets of plants grew at about the same rate, it is concluded that high-nitrogen nutrition increased the rate of multiplication of the virus.

*Population Trends of Physiologic Races of Puccinia graminis tritici in the United States from 1930 to 1939.* E. C. STAKMAN, R. C. CASSELL, AND W. Q. LOEGERING. There have been decided population shifts among physiologic races of *Puccinia graminis tritici* in the United States during the past 10 years, as determined by the percentage of uredial isolates of each race obtained from several hundred collections identified each year. Some races, such as race 38, have fluctuated greatly; others, such as race 34, increased gradually and then gradually declined (0.6 per cent in 1930; 22 per cent in 1934; and 0.6 per cent in 1939); some have declined to almost zero, notably race 36 (36 per cent in 1930 and 0.6 per cent in 1939); at least one, race 21, which constituted 7 per cent of

all isolates in 1934, was not found on wheat at all in 1939; still others, races 17 and 19, for example, have shown a general tendency to increase slowly but somewhat irregularly; and one, race 56, increased from 0.3 per cent in 1930 to 66 per cent in 1938 and 59 per cent in 1939. Indications are that temperature and other meteorologic factors may be important in these shifts. The implications of the facts in interpreting epidemics and in development and maintenance of resistant varieties are evident. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

*Potato Ring-rot Spread and Its Control by Disinfectants.* G. H. STARR. Experiments conducted in 1939 at Laramie, Wyoming, showed corrosive sublimate to be the most effective, Mercurnol relatively less effective, and acid-formaldehyde, Semesan Bel, Cinnex 20, formaldehyde, and lysol least effective as knife disinfectants for the control of spread of ring rot in potato tubers. After 2 days of healing-over, the percentage of ring rot, when compared with that in lots planted soon after cutting, was not reduced. In the treatment of potato tubers, both before and after cutting, Mercurnol gave better control of ring rot than did Semesan Bel, Cinnex 20, or hot formaldehyde. There was 65 per cent of ring rot in seed treated before cutting, and but 40 per cent in that treated after cutting. The use of whole seed gave 23 per cent of ring rot, while slightly-infected seed gave 85 per cent. The cutting knife spread ring-rot infection to the 10th and last tuber of each lot. When the eyes of healthy tubers were smeared with ring-spot inoculum, infection occurred in 45 per cent of the plants. If inoculum was merely rubbed on the sound skin between the eyes, no infection resulted.

*Treating Deciduous Trees for Chlorosis.* G. H. STARR. Chlorosis of deciduous trees, cottonwoods in particular, has been especially severe during the past few years on the University of Wyoming campus and in many other parts of Wyoming. Injection of ferric phosphate in holes bored in the trunks of trees has given highly satisfactory results. Fifty-year-old trees, as well as younger ones, have responded to the treatment. What may be the correct amount to inject for successful results is still being tested. Five grams of ferric phosphate per inch-diameter of trees has given satisfactory results, but 5 times that dosage has been equally successful and perhaps its effect may last longer. Total leaf fall has resulted from summer treatments, but green leaves soon reappeared and obtained almost normal size before fall. Early-spring treatment of trees that appeared almost dead from severe chlorosis have been rejuvenated and restored to a normal green color. Several trees used as controls are now dead. The duration of treatment effect is not yet known, but previous results have shown that benefits may be expected for 4 or 5 years after treatment. Perhaps heavier dosages will increase this period.

*Phloem Necrosis in the Ohio River Valley.* ROGER U. SWINGLE. Phloem necrosis of elm continued in epidemic form during 1939. Although no extensive survey has been undertaken, the disease has been found in Ohio, Indiana, Illinois, Missouri, Tennessee, Kentucky, and West Virginia. To date it has been transmitted only by grafting. About 80 to 99 per cent of the patch grafts made union and about a year later 82 to 85 per cent of these showed the disease. In addition to the typically chronic cases of the disease reported previously, acute cases have been observed. In the former there is a gradual decline over a 12- to 18-month period before the tree dies; whereas, in the latter, apparently healthy and vigorous trees suddenly wilt and die within 3 or 4 weeks. During the past season 2 trees that expressed typical disease symptoms in previous years showed a marked degree of recovery. Seedling elms from one of these trees were extremely variable and, although none developed phloem necrosis, a few showed mosaic-like mottling of the foliage. Attempts to induce recovery of diseased trees by applications of the minor element boron and complete fertilizers have been unsuccessful.

*Preemergence and Postemergence Factors that Influence the Infection of Barley by Covered Smut and Nigra Loose Smut.* V. F. TAPKE. These two smuts, like the other seedling-infecting smuts of small grains, invade their host during seedling growth from the seed to the soil surface. Soil conditions during this preemergence period, especially moisture and temperature conditions, long have been considered the important factors affecting infection and the incidence of smut. In line with recent results with barley covered smut and oats loose smut, it was found that cold conditions, after the seedlings have emerged, also may result in marked reductions in the incidence of the nigra loose smut of barley. Temperate conditions for 10 days, 30 days, and continuously after seedling emergence resulted in progressively marked increases of smut. In another study, distinct increases in covered smut were obtained through impeding the subterranean growth of the seedlings by tamping or deepening the soil layer above the seed or by using a heavy soil. In another experiment, the incidence of covered smut was more than double when the early growth of fully-emerged seedlings was retarded through pruning the roots.

*Control of Leaf Spot on Sour Cherries in West Virginia.* CARLTON F. TAYLOR. Four applications of lime sulphur at 3 gal. of concentrate per 100 gal. of spray material is the accepted leaf-spot-control program on sour cherries in West Virginia. An experiment was conducted in 1939 to test the efficiency of this program. Each material under test was applied to 10 young (2-year) Montmorency trees arranged in single tree plots in randomized blocks. Sprays were applied on May 10 (petal-fall), May 18, June 12, and July 14-15 (post-harvest). The retained, uninjured leaves (on 1939 wood) on July 31 and September 15, respectively, were at the following percentage levels: 5 variations of lime sulphur averaged 88.7 and 5.0; phenothiazine, 1 lb. with bentonite and hydrated lime per 100 gal., 16.8 and 0.1; Bordeaux 3-2-100, 12.5 and 15.5; Bordeaux 3-5-100, 63.0 and 39.8; Copper Hydro, 3 lb. and 2 lb. of hydrated lime per 100 gal., 79.6 and 62.1; and nonsprayed checks (average of 5 plots) 0.3 and 0.0. Differences greater than 10.5 and 10.6 are probably significant at odds of 20: 1. With the heavy infestation prevailing in this experiment, copper materials tested were much superior to lime sulphur in late-season retention of foliage.

*Problems in the Determination of Physiologic Races of *Ustilago avenae* and *U. levis*.* IAN W. TERVET. When susceptible varieties of oats were inoculated each year with different collections of oat smut, the percentage of smutted heads caused by some collections varied from year to year, while that caused by other collections remained constant for 6 years. One collection of *Ustilago levis* produced a relatively constant percentage of smutted heads on the susceptible variety Anthony for 6 years, but attacked Igold lightly the first 2 years and very heavily the last 4. In 1937 a collection consisting mainly of *U. avenae*, with a slight admixture of *U. levis*, or a hybrid between these species, produced a moderate amount of smut on Anthony, Gopher, Igold, and Black Mesdag. Spores of this collection produced on Black Mesdag were then used to inoculate these same 4 varieties in 1938 and 1939. Only Anthony and Black Mesdag became smutted, and the chlamydospores formed were characteristic of *U. levis*. Variation in these two cases can be ascribed to the selective effect of the variety from which the chlamydospores for inoculum were obtained. In the first case the host range of the collection was increased; in the second, the host range and the specific nature of the collection were changed.

*Permeability Change as a Significant Factor in Parasitism.* F. S. THATCHER. On susceptible wheat varieties *Puccinia graminis tritici* causes an increase in the permeability to water and to solutes of the host-cells of infected tissues. Resistance of Mindum wheat to race 36 is associated with an extreme localized decrease of host-cell permeability, which is considered to result in ultimate starvation of the pathogen. Increased susceptibility of Mindum wheat to race 36, as induced by narcotization, is related to increased permeability. Increased permeability precedes pectinas activity among soft rots, and is apparent in potato petiole tissue in a region beyond the zone occupied by mycelium of *Phytophthora infestans*. These facts explain certain characteristic symptoms of soft rots and late blight, and indicate an accessory rôle of permeability increase in the parasitism of the pathogens concerned. A decrease in the permeability of tissues of swede "root" near the margin of necrotic lesions caused by *Phoma lingam* was interpreted as being in accord with Brown's suggestion that a dry rot is determined by the ability of the host plant to restrict the amount of water reaching the parasite and, thus, arrest the progress of its enzyme activity at some intermediate stage. Wilt of excised tomato stems as induced by filtrates of cultures of *Fusarium lycopersici* was associated with increase in permeability of mesophyll cells to water. Wilting was not caused by death of xylem parenchyma cells associated with the conducting elements or by interference with the transpiration stream by pit closure. (McGill University. At present, Fellow Royal Society of Canada working in the Section of Plant Pathology, University Farm, St. Paul.)

*Additional Facts Regarding Bacteriophage.* ROY C. THOMAS. A nonspecific lysis, which is inactivated by heating at 56° C. for 30 minutes, has been found in many plants. When this lysis comes in contact with susceptible bacteria a change occurs resulting in the formation of a transmissible lytic principle, which is not inactivated at 60° C. and only partially at 65° C. This is believed to be the origin of the bacteriophage in plants and to function as a mechanism of resistance. These lytic principles vary with differences in cultures of bacteria used to produce them. In corn varieties susceptible to bacterial wilt, the lysis was lacking or very weak, whereas in resistant varieties it was strong. Several methods have been found effective in rendering cultures of *Aplanobacter stewarti* free of the lytic factor.

*Inheritance of Resistance to *Erysiphe graminis hordei* in a Cross between Featherstone and Nepal Barley.* J. S. TIDD. Greenhouse studies were made of the F<sub>1</sub> and F<sub>2</sub> of a cross between the barley varieties, Featherstone C.I. 1120 and Nepal C.I. 595. In addition

to being susceptible to race 6 of barley powdery mildew, Featherstone is awned, has a hulled caryopsis, and a short-haired rachilla. Nepal is resistant to race 6, is hooded, has a naked seed, and a long-haired rachilla. The  $F_2$  plants were classified for these characters, and  $F_2$  progeny tests also were made to check the  $F_2$ -mildew classification. The data indicated that resistance was incompletely dominant, heterozygous  $F_2$  plants being less resistant than the homozygous resistant plants. One main Mendelian factor is evidently responsible for the expression of resistance of Nepal to race 6. Mildew reaction also is inherited apparently independently of the other 3 character pairs studied in the cross, no linkage being found between any of the 4 factor pairs studied.

*Verticillium Wilt of Chrysanthemums.* PAUL E. TILFORD AND HARMON A. RUNNELS. Many varieties (423) of florists' chrysanthemums have been tested for resistance to *Verticillium* wilt. Those failing to develop symptoms and from which the fungus could not be isolated from the stems of plants grown in soil heavily infested with *Verticillium* were considered resistant. Roughly one-third (32.38 per cent) of the varieties proved resistant. Attempts to eliminate the fungus from cuttings by permitting them to take up fungicidal solutions have failed. Symptoms are most pronounced when the plants are in flower, and are least evident during periods of rapid vegetative growth. By roguing diseased plants when in flower, saving the first tip cuttings from the apparently healthy plants in the spring and growing the new plants in sterilized soil it has been possible to greatly reduce the amount of disease in a single season. Isolates of *Verticillium* from *Liatris*, soft maple, sugar maple, Norway maple, American elm, Japanese barberry and potato, although variable in culture, depending on cultural conditions, appear the same as isolates from chrysanthemum. Chrysanthemums, eggplants, and cinerarias were inoculated with most of these isolates. Some variability in virulence was observed but all were pathogenic.

*Control of Leaf Blights of Fig.* E. C. TIMS. Bordeaux mixture having proved ineffective in Louisiana as a control for thread blight of fig caused by *Corticium stevensii*, preliminary tests were made several years ago with some copper sulphate-lime arsenite eradicant sprays applied during the dormant season. These spray mixtures gave good results from the beginning, and in 1937 and 1938 gave almost complete control. Several trees sprayed with a copper sulphate-lime-zinc arsenite-monocalcium arsenite-fish oil mixture in 1938 remained free of thread blight during 1939, indicating that this spray mixture has a strong eradicant effect on the sclerotia of *C. stevensii*. The tests in 1939 were complicated by another leaf-blighting organism (*C. microsclerotia*), which caused very severe defoliation of many fig trees early in the season. While the above-mentioned spray mixture caused almost complete control of *C. microsclerotia* until the fig crop had been harvested in July, there was later some spread of the disease from adjoining non-sprayed trees to the sprayed area. This late infection probably was caused by sclerotia that developed in great numbers on the nonsprayed trees.

*Observations on a Noteworthy *Helminthosporium* Disease of Corn.* ARNOLD J. ULLSTRUP. The inbred line of corn Pr and two proprietary inbreds were observed to suffer from a severe attack by a species of *Helminthosporium* during 1938 and 1939. The symptoms on the leaves of Pr are characterized by numerous lesions ranging from 1 to 15 mm. in length. The lesions, generally oblong, because of partial delimitation by the veins, show faint zonation. Coalescence of lesions is common. The infected areas are at first a dead brown; later, with onset of fruiting of the fungus, becoming greenish gray. All aboveground parts are conspicuously attacked. Initial infection has been observed to take place in early summer. Under conditions of continued high humidity, fruition of the parasite is abundant and soon the entire plant is involved. Ears are attacked at any point and the mycelium may soon cover a large portion of the kernels. Morphologically, the fungus is distinct from *Helminthosporium turicum* and *Cochliobolus heterostrophus*. The symptoms also differ from those produced by these species. *Helminthosporium zeicola* appears to be morphologically similar to the species in question. Symptoms indicated on the type specimen of *H. zeicola*, as well as reports on the pathogenicity of the latter do not suggest identical species. (Cooperative investigations of the Division of Cereal Crops and Diseases and the Purdue University Agricultural Experiment Station.)

*Virus Distribution in Mosaic-susceptible and Mosaic-resistant Burley Tobacco.* W. D. VALLEAU AND STEPHEN DIACHUN. Applying a white strain of the tobacco-mosaic virus to susceptible and resistant (Ambala type) Burleys disclosed the fact that at the end of a month the distribution of virus within the plants was limited to chlorotic yellow patterns. Green areas, unless very close to yellow ones, were virus-free. The line between viruliferous and healthy tissue was more clearly marked in resistant plants than in susceptible, where invasion was more rapid. Mature, susceptible plants, inoculated at topping time

on the top and bottom leaves, respectively, developed mosaic in the suckers, and were extensively invaded in the top-inoculated leaves or were slightly invaded in the lower inoculated leaves. The remaining leaves, however, remained uninoculated for 30 days or more. After 30 days an occasional noninoculated leaf became slowly invaded along the base of the midrib, followed by a gradual spreading up and along the laterals. One striking difference between susceptible and resistant strains appears to be in the longer time required for the release of virus from infected areas of the resistant plants. There appears to be no barrier to rapid long-distance carriage of virus in highly resistant plants, once the virus is released from infected areas.

*Classification and Nomenclature of Some Phytopathogenic Species of Bacillus.* E. L. WALDEE, G. C. KENT AND I. E. MELHUS. Forty-six cultures of bacteria described as phytopathogenic species of *Bacillus* were studied according to the 1934 descriptive chart of the S. A. B. to determine their taxonomic status. From the preliminary data obtained, there emerge at least 3 well-defined groups of generic importance. Three species, formerly called *Bacillus amylovorus*, *B. tracheiphilus* and *Bacterium salicis*, constitute one group. The soft rot bacteria (24 isolates) constitute the second group. Two isolates designated as *B. lathyri* fall into a third group. Group I constitutes a generic group based on the type species, *Erwinia amylovora* (Burrill) Winslow *et al.*, 1917, and includes also *E. tracheiphila* (E. F. S.) Holland, 1920, and *E. salicia* (Day) Bergey *et al.*, 1939. Group II, comprising the soft-rot isolates, belongs to the coliform bacteria and should be classified with them in a new genus in the tribe Escherichiae (Bergey, 1939). Group III seems to contain 2 species of bacteria whose taxonomic position is still uncertain.

*Histological Studies of Storage Scab Lesions on Mature Apple Fruits.* E. A. WALKER. Two types of storage scab lesions were studied; *i.e.*, continuation of growth around the margin of the prestorage scab lesion, and new scab lesions that developed while the fruit was in cold storage. Comparative studies were made with the storage and prestorage scab lesions on 4 varieties of apples. Storage scab lesions are somewhat sunken, due in part to the crushing and disintegration of the cortical cells under the mass of the scab organism. The fungus grows profusely through the cuticle tissue in a plate-like mass of pseudoparenchymous mycelium. The hyphae continue growth around the epidermal cells and may penetrate readily into the cortical tissue to a depth of 8 to 10 cells below the epidermis. The mycelium is found in the intercellular spaces, the middle lamellae, and is rarely intracellular. There is no cork or callus formation associated with the prestorage scab lesions; however, the cells immediately below the mass of scab mycelium are badly crushed and necrotic. The cuticle increased in thickness with increased length of storage; the scab fungus was seldom observed breaking through to produce conidia.

*A Method of Reducing Clubroot Infection at Transplanting.* J. C. WALKER, MARK A. STAHHMANN AND DEAN E. PRYOR. The severest damage from clubroot (*Plasmodiophora brassicae*) on transplanted crucifers results from concentrated infection of the many rootlets that arise almost simultaneously from the lower stem and upper tap root after setting, especially since the application of water to facilitate recovery is also very favorable to infection. A weak solution of mercuric chloride used as the transplanting fluid greatly reduces infection in this zone, without injury to the host. In the search for a noncorrosive and more efficient fungicide several organic and inorganic compounds have been tested. Nothing superior to a solution of mercuric chloride in cost and efficiency has yet been secured. Use of the latter as the transplanting fluid in setting out cabbage on clubroot infested soil gave commercially significant control on both mineral and muck soils in Wisconsin in 1938 and 1939. It is suggested that this method might well be tried in other regions. (University of Wisconsin and Division Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.)

*Evidence of Passive Immunization of Plants from Curly Top.* J. M. WALLACE. Following recovery from curly top, Turkish tobacco plants showed mild symptoms and were not visibly affected by reinoculation. Leaf-hopper transmission from recovered plants resulted in severe symptoms, indicating that the virus was unaltered in virulence. On the other hand, graft transmission resulted in mild symptoms. This indicated protective substances or properties in the recovered plants, transferable by grafting. Cions taken from leaf-hopper-inoculated plants at 5-day intervals following inoculation and grafted to healthy plants showed that a period of 20 days or longer was required for the inoculated plants to develop maximum effectiveness in affording protection. Further studies showed that plants of tomato varieties that seldom exhibit any ability to recover from curly top were provided with a partial protection when infected by grafting with recovered tobacco plants. Once established in the tomato plants, the protection was retained through several serial cuttings for more than a year and was transferable to

other healthy tomato and tobacco plants. This seems to be an example of a kind of passive immunization. This phenomenon has not been previously observed in plants.

*New Facts Concerning the Plane Disease.* J. M. WALTER AND P. V. MOOK. The plane disease has killed hundreds of planetrees in the Philadelphia and Baltimore areas. Recently, cases have been found in Newark, New Jersey, South Charleston, West Virginia, Magnolia, North Carolina, and Williamsburg, Virginia. Sufficient *Platanus acerifolia* trees of different sizes have been inoculated to demonstrate that the disease is caused by the *Ceratostomella* species that Jackson believed, from limited inoculation tests, to be the pathogen. Inoculation of 12 small seedlings of *Platanus occidentalis* resulted in development of typical symptoms. Trees affected by the disease in Vicksburg, Mississippi, South Charleston, West Virginia, and Williamsburg, Virginia, have been identified as American sycamore. The initial external symptom on recently exfoliated bark areas is a dark-brown or black discoloration, usually elongated in line with the grain of the underlying wood. On bark areas retaining older layers and scales, the first noticeable symptom is an elongate depression beneath which the inner bark is darkened. Infections occur on branches high in the crown, as well as in trunks. Branches flagging during August or September on trees whose trunks showed no lesions were found to have infections approximately 1 year old. Of 45 branch infections studied to date, 26 were associated with small pruning cuts. The fungus has been transmitted experimentally with pruning saws.

*Pythium Injury of Oats.* AARON WELCH. Oats, grown under Iowa conditions, are subject to serious root injury caused by *Pythium debaryanum*. It may cause a rotting of roots and of germinating seed. Under less favorable conditions for the pathogen, only local root lesions develop, which may cause stunting, yellowing, and, later, dying of the lower leaves of the host. The causal organism was easily isolated during the seedling stage. As the season advanced, however, isolation of *Pythium* became increasingly difficult. At maturity of the plant the pathogen was not isolated. It was found, however, that numerous secondary organisms rapidly followed *Pythium* infection. The secondary organisms were not isolated in the seedling stages, when *Pythium* was most prevalent. Under temperature-controlled greenhouse conditions 32 varieties of oats were tested in *Pythium*-infested soil. Germination was poor and root injury severe at 20° C. or below. Root development of the infected plants was suppressed, top growth reduced, and the development of secondary roots retarded. Infected plants yielded only half as much as the checks in terms of grain and total dry weight. Relatively few of the 32 varieties were resistant; most of them were susceptible.

*Eradicant Sprays for the Control of Blossom Infection by Sclerotinia laxa.* E. E. WILSON. Effect of arsenite sprays on development of sporodochia of *Sclerotinia laxa* in apricot and almond trees was again studied in 1938-39. Tests, conducted in 15 orchards in 8 counties, consisted of 120 plots containing 5 to 120 trees. The spray was applied in winter before sporodochia appeared on blighted hold-over twigs. Sodium and zinc arsenites were erratic or ineffective in preventing sporodochial development, but in its ability to kill sporodochia the former showed some promise when applied after these structures appeared. Calcium arsenite, on the other hand, frequently reduced development of sporodochia 95 to 99 per cent. In some of the small plots, despite efficient sporodochia suppression, blossom infection was abundant, possibly because spores drifted in from adjacent nonsprayed trees. In the larger plots, however, the amount of disease was reduced 65 to 95 per cent. Isolations indicated that calcium arsenite killed the fungus in a majority of the hold-over twigs. In others, though the fungus was not killed throughout, it failed to develop sporodochia. Almonds, in particular, have proved very sensitive to injury by arsenites. As a consequence, an important problem confronting the successful use of this type of control method is that of preventing serious host injury.

*Comparisons of Phytomonas cerasi with Phytomonas syringae.* E. E. WILSON. Although, in morphological and cultural tests, a number of workers proved *Phytomonas syringae* and *P. cerasi* to be closely related, the two species have not been widely accepted as identical. In the present studies they were indistinguishable in their utilization of sucrose, glucose, galactose, xylose, and the sodium salts of succinic, malic, citric, and lactic acids. Their nitrogen requirements were similar in that organic sources such as asparagin and peptone supported better growth than inorganic sources, such as ammonium nitrate and ammonium chloride. Other organic nitrogen compounds utilized to some extent were glycine, leucine, and tyrosine. When inoculated into dormant plum and cherry trees both organisms produced cankers similar in size and appearance. On the basis of this and other work it is proposed that *P. cerasi* and other species (*P. utiformica* and *P. prunicola*), earlier shown to be identical with it, be identified as *Phytomonas syringae* (Van Hall).

*Comparative Values of the Fixed Coppers as Vegetable Sprays.* J. D. WILSON AND H. C. YOUNG. From 8 to 14 of the so-called fixed or insoluble copper compounds now available were applied in the summer of 1939 to such crops as ginseng, tomatoes, celery, muskmelons, and beans. Notes were taken relative to the injury caused in certain instances, and comparative yields of the variously treated plots were obtained in most of the experiments. Rather definite indications of crop and disease specificity, with reference to injury and control factors, were observed for the different compounds compared. For instance, one of the materials, which gave good control of *Alternaria* blight of ginseng, ranked much lower with reference to the *Cercospora* leaf blights of carrot and celery, and another, which injured carrots severely, caused but little injury to tomatoes.

*The Comparative Effect of Various Sulphur and Copper Sprays on Quality, Color, and Size of Sour Cherries.* H. F. WINTER AND H. C. YOUNG. In this experiment lime sulphur, flotation sulphur, Bordeaux 1-2-100, 1½-3-100, 2-6-100, and 10 of the fixed coppers were compared. The fixed coppers were applied at the rate of 3 lb. to 100, based on 25 per cent metallic copper, with 3 lb. of hydrated lime. Cherries sprayed with the sulphur sprays were perceptibly lighter in color and contained less sugar and acids than those sprayed with copper. The sulphurs did not control leaf spot. Lime sulphur, 1½ gal. to 100, stunted the leaves. In general, the Bordeaux-sprayed cherries were smaller than those sprayed with fixed copper. Copper-sprayed cherries were darker and contained more sugar and acids than those not sprayed or sprayed with sulphur. Leaf spot was well controlled by most of the copper compounds.

*The Diurnal Cycle of *Taphrina deformans*.* C. E. YARWOOD. Uniformity of the ascospores and absence of expected intermediate stages of ascus development in a given collection of curl-infected peach leaves suggested a diurnal cycle of *Taphrina deformans*. The cycle was followed by freehand and microtome sections of young material collected at different times of the day, by continuous collections of spores on slides exposed in infected trees, and by stimulating ascospore discharge from periodically collected leaves by means of the vapors from formalin-acetic acid-alcohol fixative. It was found that growth of the ascospores from the ascogenous cells began about 10 p.m. and reached full size about 3 a.m. By 6 a.m. there were usually 4 nuclei present in each ascus, and by noon the 8 ascospores were apparently mature. The maximum collection of naturally discharged ascospore groups was about 7 p.m., the minimum about 7 a.m. Exposure of infected leaves to the vapors of F.A.A. fixative induced ascospore discharge in greatest numbers in the afternoon and evening. The diurnal cycle was less marked, or was not apparent, on leaves on which ascospores had been maturing for several days.

*Sporulation Injury Associated with Downy-mildew Infections.* C. E. YARWOOD. Leaves infected with downy mildew of onions, downy mildew of spinach, and downy mildew of hops died sooner, or were more severely injured, if the fungus sporulated on the leaf surface than if such sporulation were prevented. The loss of green weight (as a quantitative measure of injury) due to a single night of sporulation was as much as 48 per cent of the original green weight for onions, 48 per cent for spinach, and 11 per cent for hops. The transpiration of leaves on which sporulation had just occurred was about 29 per cent less than that of nonsporulating infected leaves for onions and 28 per cent less for hops. The respiration of leaves on which sporulation had just occurred was not consistently different from that of nonsporulating infected leaves, but was about 47 per cent greater than healthy leaves on a green-weight basis, and amounted to about 0.23 mg. carbon dioxide per gram dry weight of leaves per hour in darkness at 19° C. Neither disturbed transpiration nor disturbed respiration is believed responsible for the sporulation injury observed, and the cause has not been determined.

*Relative Susceptibility of Young Pine Trees in Artificial and Natural Stands to Invasion by Fungi and Bacteria.* H. H. YORK. Investigations over a period of 10 years in forest plantings of white, red, and Scotch pines indicate that there is a very definite relation between the way in which trees are set in the ground and their susceptibility to infection in the root crown by fungi and bacteria. These studies, thus far, show that trees, established by natural seeding, are far less susceptible to invasion by these organisms.

*Resistance of Tomato Varieties to Blossom-end Rot.* P. A. YOUNG. With 4978 single-stem tomato plants per acre, serious commercial loss resulted from an average of only one rotted fruit per plant. Extensive field tests at Jacksonville, Texas, from 1937 to 1939 showed large variation in resistance to blossom-end rot among different tomato varieties and their selections. Most selections of Marglobe, Break O'Day, and Pritchard were resistant. Varieties tested were classified in 3 groups based on the range in average numbers of fruits with blossom-end rot per plant. The resistant group with 0 to 0.8

fruits with blossom-end rot per plant included Blair Forcing, Break O'Day, Globe, Long Calyx Forcing, Marglobe, Marhio, Michigan State, Norton, Pritchard, Sureset Forcing, and Tennessee Red. Moderately susceptible group with 0.9 to 2.0 fruits with blossom-end rot per plant included Baltimore, Century, Early Baltimore, Glovel, Grothens Red Globe, Gulf State Market, Illinois Baltimore, Kanora, Louisiana Pink, Marvann, Marvel, Newport, and Sweetmeat. Very susceptible group with 2.1 to 6.5 fruits with blossom-end rot per plant included Browns Special, Buckeye State, Globelle, Illinois Pride, Louisiana Dixie, Louisiana Red, Prairiana, Riverside, and Rutgers. Commercial strains resistant to blossom-end rot were selected from Rutgers and other valuable varieties.

## RELATION OF WOUNDS TO INFECTION OF AMERICAN ELM BY CERATOSTOMELLA ULCRI, AND THE OCCURRENCE OF SPORES IN RAINWATER

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### INTRODUCTION

Reports indicate that *Ceratostomella ulmi* (Schwarz) Buisman, causal fungus of the Dutch elm disease, may fruit in various places on field and planted elm under natural field conditions. In Europe, Fransen (5), Roepke (12), Wollenweber (20), and others observed fruiting structures of this fungus in maternal galleries and pupal cells made in diseased elm by certain scolytid beetles. Buisman (3) saw perithecia of *C. ulmi* that had developed "in the bark" of a standing diseased elm. In England, Peace (10) reported *C. ulmi* fruiting on dead wood in "sheltered places such as cracks between bark and wood," but he added that such fruiting was not commonly seen. May and Gravatt (7) stated that, under very favorable conditions, spores were formed on the cut surfaces of diseased trees and stumps and in insect tunnels in the wood. Later, May (8) observed coremia on the ends of logs cut from diseased trees and under the loose bark of old logs lying on the ground.

In field observations in and near New York City during the past 4 years the writers found that coremia of *Ceratostomella ulmi* are often produced in more or less exposed places on elms infected by the Dutch elm-disease pathogen and on decadent trees, apparently not infected by this fungus. Coremia were seen on the outer surface of the inner bark and they protruded into the space created by the loosened and partly raised outer rough bark. Sometimes this outer bark was sloughed off so that the coremia were fully exposed. Coremia were observed in the openings into leopard-moth galleries, and in scolytid entrance holes on diseased trees. They developed in scolytid feeding wounds on twigs taken from healthy trees in the field and held in glass moist chambers in the laboratory. Also, they developed frequently in artificial wounds and in scolytid feeding wounds made on experimental trees held under high moisture conditions in the greenhouse.

<sup>1</sup> The writers are indebted to Dr. D. S. Welch, for suggestions concerning experimental procedure, and for critically reading the manuscript. Thanks are due Dr. W. H. Rankin, New York State Department of Agriculture and Markets, for helpful cooperation in some of the field work; and the Boyce Thompson Institute for Plant Research for making available laboratory space and equipment.

Several workers have suggested the possibility of wind dissemination of the spores of *Ceratostomella ulmi* in the field, but all were doubtful of its importance (2, 5, 7, 8, 10, 12, 20). Smucker (14), in the United States, reported that spores could be dislodged from cultures and carried for some distance by air currents in the laboratory, but attempts by Fransen (6), in the Netherlands, to dislodge spores by means of air currents were unsuccessful.

The writers' experiments showed that spores were sometimes dislodged and carried by air currents. This seemed dependent on the age and moisture content of the cultures used, and on the velocity of the air currents (the velocities were measured with an anemometer). The so-called "Cephalosporium" stage of *Ceratostomella ulmi* was present on all cultures used in this work, and it was noted that bits of mycelium were almost or quite as easily torn away from such cultures as were the spores themselves. The results show further that, always, only a few spores are dislodged regardless of the conditions prevailing in the different experiments. Available data are insufficient to determine the optimum conditions for dislodgment of spores by means of dry air currents. Further work is needed to help clarify this question.

Wollenweber (20) suggested that the Dutch elm-disease fungus might be disseminated by water. The writers found that large numbers of *Ceratostomella ulmi* spores could be dislodged from cultures by means of atomized water. The cultures from which spores were dislodged contained both conidia and conidiophores growing on sterilized elm twigs. Dislodged spores were carried in small droplets of water and many were caught on nutrient agar in dishes distributed at distances of immediately below to 2 to 3 feet away from the source of inoculum. Comparison of the results obtained from attempts to dislodge spores by means of dry air currents and by means of water showed that, in the laboratory experiments, water was decidedly more effective as a disseminating agent of this fungus. Because of the ease with which spores were dislodged by water it appeared that rainwater might wash them from the fruiting structures of *C. ulmi*, formed in more exposed places on elm in the field. Therefore, rainwater was collected from diseased and apparently healthy field elms and tested for presence of this organism.

#### ISOLATION OF CERATOSTOMELLA ULMI FROM RAINWATER

During the summer months of 1937 and 1938, rainwater was collected from a total of 32 diseased and 8 healthy elms located in Westchester County, New York.<sup>2</sup> The water was caught by means of special apparatus attached to the tree trunks near their bases. One to 11 liters of water were caught and collected from each tree. This water was brought to the laboratory and centrifuged. Small samples of the material concentrated on the bottom of the centrifuge bottles were withdrawn with a sterilized pipette and mixed with nutrient agar in Petri dishes. After incubation at room temperature

<sup>2</sup> George E. Thompson, now of the Department of Plant Pathology and Plant Breeding, University of Georgia, did the necessary field work during the summer of 1937.

for several days the dish cultures showed that *Ceratostomella ulmi* was present in water collected from 9 of 32 diseased trees, but was not present in the water collected from the 8 apparently healthy trees. The number of *C. ulmi* colonies obtained per 2 cc. sample of centrifuge concentrate ranged from 1 to 39. Since this sample represented only a small part of the total volume of concentrate per liter of water, it is obvious that considerable numbers of spores were present in water from some of the trees.

Possible sources of *Ceratostomella ulmi* spores found in rainwater, which was collected from diseased trees are as follows: (a) fruiting structures formed in more or less exposed places; (b) frass pushed out by insects during construction of their egg galleries; (c) spores deposited by *C. ulmi*-infested insects, such as *Scolytus multistriatus* Marsh., *Hylurgopinus rufipes* Eich., *Saperda tridentata* Oliv., *Magdalais barbita* Say, and *M. armicollis* Say, during their visitations to and activities in diseased trees; and (d) in undetected perithecia, although as far as known such fruiting structures have never been seen on diseased trees in the field in the United States.

#### INTRODUCTION OF CERATOSTOMELLA ULCI SPORES INTO WOUNDS ON AMERICAN ELM

During the past 4 years many kinds of wounds were tested as infection courts, on different parts of 3- to 4-year-old budded elms (*Ulmus americana* L.) potted in pails. Based on parts of trees injured and on the extent of the injuries, 4 classes of wounds were tested.

*Class 1.*—Wounds which penetrated but did not extend through the cortical layers. Such wounds were made in bark of (a) current-year shoots, (b) 1-year-old branches, and (c) 2-year-old bark of trunks. The wounds were made by cutting transversely into the bark with a knife, then stripping the outer bark downward from the cut so that the inner cortical layers were exposed. When such injuries were made the wood underneath was never injured or exposed.

*Class 2.*—Wounds that exposed the outermost surface of the wood in roots. Longitudinal incisions were first made with a knife in the bark of branch-roots and of main-roots, care being taken that such incisions did not reach the wood. The bark on one side of the incision was pried loose exposing the uninjured surface of the wood and the spore suspension was then introduced under the bark with an atomizer. Inoculum was thus applied directly to the uninjured surface of the wood.

*Class 3.*—Wounds that exposed and injured the wood of roots, stems, and branches. Such wounds consisted of (a) slanting cuts made with a knife on trunks and branches; (b) *Scolytus multistriatus* feeding wounds; (c) fresh pruning wounds; (d) old pruning wounds; (e) small branches twisted at their bases so that the bark was split and the wood injured and exposed; (f) broken shoots and branches; and (g) cuts made with a one-half-inch wood chisel on the roots.

*Class 4.*—Wounds that exposed and injured the xylem elements of newly formed or forming parts of green and succulent shoots and leaves.

Such wounds were made by (a) vigorously whipping together the tree-tops so that leaves and green, succulent shoots were abraded and broken; (b) detaching leaves so that leaf traces were exposed; and by (c) puncturing leaf midribs with a needle.

With the exception of root wounded trees included in class 3, 4 trees were used to test each kind of wound on a given date. At least 1 test was made of each kind of wound during the active growth period<sup>a</sup> of the trees, and repeated once or twice on different groups of trees after terminal growth had ceased. This was done to study the effect of introducing *Ceratostomella ulmi* into different kinds of wounds during different stages of tree growth. Each kind of wound on aboveground parts was tested on 11 to 28 trees in experiments done during the 4-year period. The numbers of wounds per tree ranged from 5 to 15, depending on the kind and means by which they were made.

The relation of humidity to infection of elms by *Ceratostomella ulmi*, through wounds on aboveground parts, also was studied.

Root wound tests were started early in April, when the buds had begun to swell, and repeated on different groups of trees (3 to 12 trees per group) at intervals of 1 week to 1 month until early November. Wounds were made on the roots usually by piercing the soil around each tree several times with a one-half-inch wood chisel. The number of such wounds per tree was not determined.

Three methods of applying the inoculum were used: 1. *Ceratostomella ulmi* spores were suspended in water and atomized directly on wounds. 2. Spores were dislodged from cultures by means of water spray. In this method the spray from an atomizer containing water was forced past coremia and conidiophores that developed on autoclaved elm twigs previously planted with the fungus. Twig cultures were held above the trees and 12 inches outside the crown periphery. As the water spray came in contact with the fruiting structures, spores were dislodged and carried in droplets to wounds on the trees. 3. Root wounds, included in class 3, were exposed to inoculum by infiltrating 500 cc. of a water suspension of spores into the soil around each potted tree. Care was taken, when any one of the methods was used, to distribute approximately equal amounts of inoculum to each tree. Except for old pruning wounds, the inoculum was always introduced into the wounds as soon as they were made.

Percentages of wilt and dieback were recorded at short intervals for each of the trees until they were cut, which was usually in the fall of the year they were inoculated. Some were held for observation in the following year after which they were cut. When cut the trees were carefully examined for

<sup>a</sup> The term "active growth period," as used in this paper, denotes that period beginning (in potted trees used in these experiments) when the new shoots reach 2 to 4 inches in length and continuing until extension in shoot length ceases. This period begins 4 to 5 weeks after the leaf buds begin to open and ends about 2 months later. It is during this period that American elms appear to be most susceptible to infection and invasion by *Ceratostomella ulmi*. However, in trees becoming infected during the latter part of this period there is a definite tendency toward less extensive invasion by the fungus.

the brown discoloration commonly caused by *Ceratostomella ulmi*. The recovery of this fungus from discolored wood was used as the criterion of infection.

The results obtained from introducing *Ceratostomella ulmi* spores into wounds made on small elms are given in table 1. In general, these results agree with those reported by other workers (1, 4, 9, 13, 15, 17, 18, 19). The data for each wound class are discussed separately.

*Class 1.*—Infection was not obtained in trees by introducing spores into wounds made in cortical tissues that did not reach the wood. Radulescu (11) concluded, with some reservation, that infection could result from introducing the Dutch elm-disease fungus into bark wounds that did not injure the wood. The writers observed that injuries to the bark sometimes resulted in splitting that produced fissures in the inner bark tissue beneath the wounds soon after they were made. This splitting seemed partly attributable to pressure exerted on the injured bark due to normal radial growth of the injured plant part. Data in table 1 show that infection occurred in 5 trees; but in each of these cases it was observed that the inner-bark tissue covering the infected wood had been split. Apparently the fungus had, in these cases, reached the wood through fissures produced by splitting. In the remaining trees the inner bark underneath wounds did not split, and the fungus did not penetrate the intact tissue, at least not sufficiently to reach and establish itself in the wood.

The wounded bark tissue in each wound was excised and planted on nutrient agar in Petri dishes when the trees were cut. *Ceratostomella ulmi* was recovered from very few of these tissue plantings. Recovery of the fungus showed that it lived at least 3 months in the bark and during this time it apparently was unable to penetrate to the wood.

*Class 2.*—The number of trees that became infected through direct application of spores to the uninjured surface of the wood in roots was high, although the infected trees were not always invaded to the same extent. All trees inoculated on April 25, before the active growth period began, were infected, but only 1 was extensively invaded, the others showing mere traces of discoloration in the wood at the inoculation points. All trees inoculated during their period of active growth (June 2 and July 13) were infected and three-fourths were extensively invaded. Of those trees inoculated on August 22, which was about 5 weeks after terminal growth had ceased, 1 became infected and developed only a trace of discoloration.

*Class 3.*—With the exception of old pruning wounds, other wounds in this class readily admitted *Ceratostomella ulmi*. Old pruning wounds, having aged 3 months or more when the fungus was introduced into them, apparently had healed sufficiently during this time to entirely prevent entrance of the fungus. The incidence of infection through favorable wounds appeared to depend somewhat on the inoculation method (Table 1). The growth stage of trees at time of inoculation exerted a marked effect on the extent of subsequent invasion. The effect of the growth factor is discussed elsewhere in this paper.

TABLE I.—Results of introducing *Ceratostomella ulmi* into wounds made on American elm

Wounds*		Date; numbers of trees inoculated and infected					
Class	Kind	1935	1936	1937	1938	May 27	Aug. 28
1	Green bark			June 14 4/0	Aug. 5 4/2c	4/0	4/0
	One-year-old bark			4/0	4/1c	4/0	4/0
	Two-year-old bark			4/2c	4/0	4/0	4/0
2	Wood of roots exposed but not injured			July 13 4/4	Apr. 25 4/4	June 2 4/4	Aug. 22 4/1
	Slanting cuts	June 24 4/3	Aug. 12 4/3	June 12-14 4d/4	July 26 4d/2		
	Scolytid feeding			4d/3		May 13 4/4	
3	Fresh pruning	4/4	4/2	4d/3	3d/2		
	Old pruning	4/0	4/0	4/0	4d/4		
	Basal, twisted branches	July 16 4/3	July 30 4/3	Sept. 9 4d/2			
	Broken branches	4/3	4/4	4/4	4d/1	4/1	4/3
					4d/1	4d/2	
					1937		
Wood of roots exposed and injured*	Apr. 6	Apr. 17	May 4	May 25	June 18	July 13	Sept. 1
	4/2	4/1	4/3	4/4	3/3	8/8	4/4
						12/11	11/8

TABLE 1.—(Continued)

Class	Wounds <sup>a</sup>	Date; numbers of trees inoculated and infected <sup>b</sup>					
		Kind	1935	1936	1937	1938	
4	Whipped foliage	July 16 4/0	July 30 4/1	Sept. 9 4/10			
	Leaf scars and traces			June 24 4/3	Aug. 12 4/2	June 14 4/1	Aug. 5 4/4
	Punctured leaf mid- ribs	4/0	4/1	4/0	4/1	4/0	4/1

<sup>a</sup> See text for description.<sup>b</sup> Inoculation was by method no. 1 (see text) except as otherwise noted below by footnotes d and e. Inoculated and infected trees expressed as a fraction: numerator = trees wounded and inoculated; denominator = trees infected. The recovery of *C. ulmi* from discolored wood was used as the criterion of infection.<sup>c</sup> Injured bark had split due to radial growth; see text for explanation.<sup>d</sup> Inoculated by means of method no. 2; see text for description.<sup>e</sup> Inoculated by means of method no. 3; see text for description.

*Class 4.*—Data given in table 1 show that leaf traces were favorable for the entrance of the fungus. Infection very infrequently resulted from spores introduced into punctures made in leaf midribs, and into abrasions made on succulent parts of growing shoots. Trees infected from spores introduced into wounds of this class never were extensively invaded; the fungus always remained localized and none of the trees wilted.

Non-wounded trees were inoculated and included in each experiment as checks. The 3 methods of inoculation were tested on such trees, but none of these check trees became infected.

Briefly summarized, the data show that infection never resulted in trees through non-wounded surfaces. Infection was not obtained through injured cortical tissues that did not reach the wood, although *Ceratostomella ulmi* was reisolated from injured bark tissue several months subsequent to inoculation. Infection was obtained through the intact surface of freshly exposed wood, but invasion was more extensive in trees inoculated during their active growth period than in those inoculated before or after this period. Infection was easily obtained by introducing spores into wounds that exposed and injured the xylem elements of roots, stems, branches, and mature parts of new shoots. Infection sometimes resulted from introducing spores through injuries on leaf midribs, leaf traces, and through injuries on succulent parts of young shoots, but the fungus remained localized and never caused wilt. Wounds particularly favorable for the entrance of *C. ulmi* were: root wounds that extended into the wood; slanting cuts on trunks and branches; fresh pruning wounds; broken, and twisted branches; and *Scolytus multistriatus* feeding wounds.

The 3 methods of inoculation used were generally successful, although the direct method of introducing spores into wounds on aboveground parts by atomizing them with a water suspension of spores was somewhat more sure than the method whereby spores were dislodged from cultures with and carried to wounds by a water spray. Data in table 1 show that the incidence of infection in trees inoculated by the second method sometimes was not so great as in those inoculated by the first method. This difference was more obvious when placed on the basis of total wounds involved. Undoubtedly this was due to the accumulation of a greater number of spores per wound by the first method, resulting in a more pronounced effect from the mass attack. Inoculation accomplished by the third method of placing spores in the soil around wounded roots (Class 3) was particularly successful. This method resulted in a high incidence of infection and usually in extensive invasion, although the extent of the latter depended largely on the growth stage of trees at the time of inoculation.

It has been demonstrated that spores of *Ceratostomella ulmi* may be introduced into American elm, through wounds that expose xylem elements and thereby cause infection. Also it has been shown that spores of this pathogen are sometimes present in the "run-off" water from diseased elms. Since wounds are commonly present on various parts of field and planted elms, it

seems obvious that spores may sometimes be introduced into them by means of water. *C. ulmi* spores suspended in water and infiltrated into soil around the roots of potted elms caused infection through wounds made on the roots as long as 2 weeks after the spores were originally placed in the soil. Furthermore, Verrall (16) reported that *C. ulmi* survived sparingly in non-sterilized forest humus *in vitro* as long as 3 months.

#### FACTORS INFLUENCING THE OUTCOME OF INOCULATION

##### Stage of Growth

Infection was obtained by introducing spores of *Ceratostomella ulmi* into the soil around wounded roots at any time from April 2 to November 3, inclusive (Table 1, Class 3). The percentage of trees becoming infected was greatest in those inoculated during the period between May 25 to September 1, inclusive. Further, there was great variability in the extent of invasion resulting from inoculation and infection at different times between April 2 and November 1. Trees inoculated before buds began to swell and up to the time new shoots had reached 1 to 3 inches in length (the latter figures represent the maximum length of new shoots on trees inoculated on May 4) were mostly infected but seldom if ever severely diseased. Discoloration in the wood of such trees caused by *C. ulmi* was always scanty and usually in the annual ring formed in the preceding year. The invasion, which followed inoculation and infection on and after May 25 until terminal growth of shoots had ceased (terminal growth was completed by July 1 to 15), was extensive and most of the trees were severely diseased; this is based upon leaf wilt, dieback, and fungous discoloration. Trees inoculated within the period between cessation of terminal growth and early September often became extensively invaded, as shown by fungous discoloration, but they died back very little and most of them grew well during the following year. Most of the trees inoculated in October and early November were infected but the invasion resulting was incipient.

Data given in table 1, classes 3 and 4, show that inoculation of trees on different dates did not significantly affect the incidence of infection through different kinds of wounds, except for root wounds. For example, spores introduced into fresh slanting cuts on different trees, on June 24 and August 12, by atomizing them with spores, resulted in equal numbers of infected trees on the 2 dates. Infection results obtained from introducing spores into other kinds of wounds in these classes, by the same method, are similar and they substantiate the statement that the date of inoculation, or rather the growth stages (within the date limits of these inoculations) did not greatly influence the incidence of infection. However, there was great variability in the extent of invasion following inoculation and infection of trees during the different months. In general, extreme invasion, followed by severe dieback, resulted from introducing *Ceratostomella ulmi* into any kind of wound on aboveground parts (except bark wounds that did not reach the

wood, wounds on leaves, those on succulent parts of green shoots, and exposed leaf traces) during the active growth period of the trees. Subsequent inoculations done until the end of August generally resulted in less extensive invasion and the trees seldom died back. Invasion of such trees also was variable, as shown by discoloration, and depended to some extent upon the kind of wound used as an infection court. Invasion was more extensive when the inoculum was introduced through slanting cuts in trunk and branches, broken branches, fresh pruning wounds, and *Scolytus multistriatus* feeding wounds than it was when the inoculum was introduced into wounds made by twisting branches, into leaf traces, wounds on leaves, and into other wounds which exposed but did not injure the xylem elements.

### Humidity

In all wound experiments, except those involving root wounds, some trees from each wound-group were placed on greenhouse benches immediately following inoculation, and their foliage and branches were always kept dry. Other trees from the same groups were held in a greenhouse moist chamber for 10 days immediately following inoculation and then removed and placed on greenhouse benches; thereafter, their foliage and branches also were kept dry. The relative humidity maintained in the moist chamber ranged predominantly from 85 to 98 per cent, while that of the greenhouse, with ventilators constantly open, ranged predominantly from 40 to 80 per cent. Wounded tissue on trees held in the moist chamber remained moist throughout the 10-day treatment, while that on trees in the open greenhouse rapidly became dry. Infection was obtained more frequently in trees placed in the moist chamber than in those exposed constantly in the open greenhouse. This difference was evident in all experiments involving the introduction of *Ceratostomella ulmi* spores into different kinds of wounds, on aboveground parts, which were most suitable for entrance of the fungus. These inoculations were made from May through August. The moist-chamber treatment did not appear to influence the extent of invasion, since trees becoming infected under this condition were invaded to about the same extent as those held under drier conditions.

The moist-chamber treatment very markedly affected the ability of *Ceratostomella ulmi* to fruit in the wounds. On trees exposed in the moist chamber, coremia were present in all types of wounds made on aboveground parts, except those made on leaves and on succulent parts of green shoots, but there were none in those wounds on trees held constantly in the open greenhouse.

### RELATION OF WOUND HEALING TO INFECTION

It has been shown that infection may result from introducing *Ceratostomella ulmi* spores into many kinds of wounds freshly made on small elms. To study the relation of wound healing to infection, spores were introduced into wounds after varied periods of aging and healing. Three kinds of wounds were used: (a) slanting cuts made with a knife on stems and on

branches; (b) feeding wounds made by surface-disinfected *Scolytus multistriatus* beetles; and (c) cuts made on roots by piercing the surrounding soil with a  $\frac{1}{2}$ -in. wood chisel. After the trees were wounded they were held outdoors to allow their wounds to heal under more nearly natural conditions. Trees having wounds on aboveground parts were moved into a cheesecloth-screened greenhouse just before inoculation, where they remained until cut and examined. Each tree was carefully examined before being inoculated to make sure that wounds other than those made for the test were not present.

Some trees with beetle wounds and some with knife wounds were brought to the greenhouse and inoculated on the day wounds were made. Since beetles fed 3 days before being removed from the trees, some feeding wounds were as much as 3 days old when the first trees were inoculated. Thereafter, different trees with wounds were brought to the greenhouse and inoculated at intervals of 3, 6, 13, and 27 days subsequent to the day they were wounded. Trees having wounds on aboveground parts were inoculated by atomizing a water suspension of spores directly upon the wounds. Following inoculation, each group of trees was divided; some of the trees were placed on greenhouse benches where their branches and foliage were always kept dry; the others were held on benches in the same greenhouse and their branches and foliage sprinkled lightly with water twice daily for 7 days, after which they were treated like those always kept dry.

By means of the soil-infestation method, some of the root-wounded trees were inoculated on the day wounds were made; others at intervals of 1, 3, 7, 14, and 30 days subsequent to being wounded. After inoculation these trees were held in an outdoor cage made of wire screen.

All trees were wounded and inoculations completed within the period of their active growth. Notes on wilt and dieback in each tree were taken at short intervals. The trees were cut in the autumn of the year they were inoculated, and each tree was examined in detail to ascertain the presence or absence of fungous discoloration. The isolation of *Ceratostomella ulmi* from discolored tissue was used as the criterion of infection.

The results definitely show that, for knife wounds and for beetle-feeding wounds, the chances for infection became progressively less as the interval allowed for healing of wounds was increased. All trees inoculated during the first 6 days were infected and most of them were extensively invaded, while others inoculated at the end of the 13-day interval were likewise all infected but only 1 was extensively invaded. Infection was slight following the introduction of spores into wounds after 27 days. The fungus gained entrance through only 20 per cent of the wounds into which spores were introduced at this time; the resulting discoloration from which *Ceratostomella ulmi* was isolated showed that the fungus had progressed not more than 1 to 2 cm. in the tissue around these wounds. Briefly, it appears that beetle and knife wounds made on small trees require somewhat more than 27 days for healing to entirely prevent infection. Infection followed by extensive invasion may be expected occasionally from the introduction of spores into 2-week-old wounds and frequently when the wounds are less than 2 weeks old.

The effect of sprinkling was noticeable only in trees inoculated at the end of the 27-day interval. Knife-wounded trees that were not sprinkled were not infected, while some of the sprinkled ones were.

The results obtained from introducing *Ceratostomella ulmi* spores into root wounds of different ages were somewhat similar to those for wounds of different ages on aboveground parts. Root wounds up to 1 week old markedly favored fungal entry; all trees were infected and most of them were extensively invaded. Trees with 2-week-old wounds were mostly infected but none were extensively invaded, while trees with 1-month-old wounds were not infected.

#### SUMMARY

Field observations show that coremia of *Ceratostomella ulmi* are often produced in various places on field and planted elms infected by this organism, and on decadent elms apparently not infected by this fungus. Coremia in some of these places are sometimes fully exposed to the outside.

In laboratory experiments, *Ceratostomella ulmi* spores were easily dislodged from fruiting structures by means of a water spray, but spores were not easily dislodged by means of dry air currents.

During the summer months of 1937 and 1938 rainwater was collected from a total of 32 diseased field elms; water from 9 of these yielded *Ceratostomella ulmi*.

Infection was easily obtained in potted American elms as a result of introducing *Ceratostomella ulmi* spores into fresh wounds that extended into the wood of roots, trunks, and branches. Infection also was obtained when inoculum was applied to the surface of wood freshly exposed but uninjured. Infection sometimes was obtained when spores were introduced into leaf traces, and into injuries on leaf midribs and succulent parts of newly formed or forming shoots; but the resulting invasion was always slight, and the trees did not wilt. Infection did not result from applying spores to non-wounded surfaces; neither did infection result from introducing spores into cortical wounds that did not reach the wood.

Data are discussed showing that the aging and healing of wounds made on potted elms had a marked effect upon the entrance of *Ceratostomella ulmi*.

Potted trees, inoculated at any time within their active growth period, were extensively invaded; many of them died back considerably. Trees inoculated before this period frequently became infected, but invasion by the fungus usually was slight. Trees inoculated after this period usually became infected, and some were extensively invaded; these died back very little or not at all.

Infection occurred more frequently in trees, inoculated through wounds on aboveground parts, which were held under high moisture conditions, than in other trees similarly inoculated but held under drier conditions. Constant high moisture conditions favored the development of coremia in wounds.

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ECOLOGICAL SPECIALIZATION IN THE STEM- AND BULB-INFESTING NEMATODE, DITYLENCHEUS DIPSACI  
VAR. AMSINCKIAE<sup>1</sup>

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INTRODUCTION

Steiner and Scott (10) have reported for various points in California the occurrence of a nematosis of *Amsinckia intermedia* Fish. and Mey., a very

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common annual herbaceous wild flower of central California. The mature-plant symptoms are described as "greatly enlarged fruits, transformed by the parasite into obvious galls." It is stated that "All portions of the plant above ground may harbor the nema." Observations made by the writer in the spring of 1933 and subsequently have disclosed that up to the end of the growing season there is no tissue infestation, except in the fruits. Leaves, bracts of various kinds, and stems are entirely free, except for occasional migratory larvae found only on the surface. This evidence of marked tissue specialization, unusual for *Ditylenchus dipsaci*, stimulated research into the nature of the relations of the nematode to the host plant. Consequently, in the spring of 1936, infested plants were studied in every stage of development from emergence to complete maturity. The results of these studies are here reported. Figure 1 illustrates the typical symptoms of the disease in the mature plant.



FIG. 1. Typical galls in the fruits of *Amsinckia intermedia* caused by the nematode *Ditylenchus dipsaci* var. *amsinckiae*. At *x* is shown a normal mature fruit with normal seeds. The great enlargement of the diseased fruits is manifest.  $\times 1$ .

#### LITERATURE REVIEW

There are 316 species of plants representing 40-odd families, listed as hosts of *Ditylenchus dipsaci*, in the files of the Division of Nematology, U. S. Department of Agriculture. The general symptoms, applicable to all its hosts are "more or less localized deformations of stem and leaf tissues giving rise frequently to separate or confluent galls, especially on dicotyledonous

plants" (Goodey, 7). The gall tissue or gall-like tissue is pronounced in some host plants, such as *Taraxacum* and *Hypochaeris* (2, 4), where it is found in both mesophyll and veins of the leaves; in clover (8, 9), alfalfa (3), and other legumes, in which stems are likewise swollen; in strawberry (8), where leaves, stems, and fruits are often greatly swollen and distorted; in *Narcissus* and hyacinth (7), where the swellings early occur as somewhat yellowish "spikkels" that can be felt between the thumb and fingers and that then chiefly characterize and differentiate the disease from other yellowing diseases. This symptom, however, is conspicuously lacking in some, even severely infested, host plants, e.g., garlic and parsley (5, 6), in which the recognizable symptoms are dwarfing of the plant and necrosis of heavily infested tissues.

In no case has there been reported evidence of any sharp selectivity, by the nematode, of particular tissues for infestation. The infective nematodes in a favorable environment enter any succulent tissues, and, without much migration there, forthwith begin their parasitic existence and promptly reproduce their kind. In some host plants, there is a certain degree of selection of the floral organs for the late-season propagation of the over-wintering generation of nematodes. This applies to *Hypochaeris radicata* L. and *Taraxacum officinale* Weber (2), in which a distortion becomes evident at the base of the inflorescence, where the nematodes are present in abundance. From this region, some of the larvae actually enter the seed capsule, where they lie adjacent to the seed, later to become wind-disseminated. But, even in these hosts, considerable multiplication of nematodes occurs within infested leaves.

#### THE DISTRIBUTION OF NEMATOSIS OF AMSINCKIA

The nature of the relation of any parasite to its host bears importantly on the dissemination and geographic range of the parasite and the disease induced by it. Incidence of nematosis of *Amsinckia* is of a scattered distribution. Steiner and Scott (10) report that up to September, 1934, the disease had been seen only from Winters, Woodland, Planada, and Monticello, California, loci confined to the interior valleys. The writer has since found it near Dixon, in the Sacramento Valley. The most marked infestation, however, was observed in a limited area beside the highway 15 miles south of San Jose, in the Santa Clara Valley. Here the degree of infestation every year ranges from that of a few scattered marginal plants to almost 100 per cent of those occupying the center of the area. Heavy stands of *Amsinckia* one-half mile north are entirely free, and, for a distance of 24 miles south, no further infestation was observed.

Nematosis of *Amsinckia* apparently is not widespread through the range of the host plant, but is limited to relatively small infestation foci of unknown origin. Obviously, the mode of dissemination of the parasite in this host does not facilitate its rapid and continuous spread.

THE HOST-PARASITE RELATIONSHIPS OF DITYLENCHUS DIPSACI  
VAR. AMSINCKIAE

As stated by Steiner and Scott (10), infested fruits are filled with nematodes in every stage of development. Most of the adults are dead by the end of the growing season; the majority of the eggs are hatched. The contents of mature, hard, and somewhat dry infested fruits consist of a dry cottony mass of immobile larvae only partly filling the gall cavity. Within 30 minutes after moistening, the swollen mass of larval worms becomes active. The writer has estimated by aliquot-part methods that an average-size gall may contain in round numbers 180,000 nematodes. Sixty per cent of these are in the resting stage of development, mostly living; 34 per cent are younger larvae, all inactive and probably dead; and about 2 per cent are dead adults. Of 50 adults picked at random, 62 per cent were females and 38 per cent males. The random gall for this count had been 5 months on a laboratory shelf.

In nature one might expect a like reactivation of dormant larvae during moist weather. Following rain or during heavy fog, when the dried plant has absorbed considerable moisture, many of the dormant nematodes regain activity. Some of the galls are open at the top or are slightly cracked laterally, thus permitting many larvae to find their way to the surface, and thence down the stem. Numbers of them have been found among the leaf hairs along the stem, in leaf and branch-stem axils, in undeveloped flowers, and at times even within the hollowed dry stem beneath the inflorescence. During the summer, the dead plants fall to the ground. Most of the nematodes thus reach the soil on the spot, still protected during the summer and succeeding winter by the tough gall tissue.

As a rule only a small proportion of the flowers are infested. The plant, therefore, matures a large crop of normal seed. These likewise fall to the ground within the zone of infestation. Thus, for the next season, there has been provided a nearly normal yield of host-plant seed and an abundance of overwintered larvae ready to enter upon their parasitic existence. Here we have evident a highly specialized type of parasitism—one in which the population of the host plant is not endangered, yet the conditions are ideal for the propagation of a continuous population of the parasites.

## Primary Infestation

In the first studies on this problem, seed of *Amsinckia intermedia* were germinated in Petri-dish moist chambers, and large numbers of infective larvae were released among them. Even after several days, no invasion of the young seedlings could be detected. Larvae were to be found on the surface of the seedlings, at the junction of the two cotyledons and in the region of the young growing point, but never within the tissues. Thus, unless some phase of the environment was distinctly adverse to infection, primary penetration of the host plant did not occur in the same manner as

with other strains of *Ditylenchus dipsaci*—as in *Hypochaeris* and *Taraxacum*, for example (2), or in clover (9) and alfalfa (3). In all of these, primary penetration takes place even in the cotyledons of the very young seedlings and, subsequently, into the interior of developing leaves and petioles.

In the early spring of 1936, the known center of heavy infestation south of San Jose was searched for young seedling plants of *Amsinckia*. On January 26, a dozen or so plants varying from 3 to 6 inches high were removed from the field and transplanted to pots in the University greenhouse at Berkeley. These grew well and, subsequently, they showed typical cases of infestation in the inflorescence. With the first collection, some of the plants were carried in moist jars to the laboratory. No evidence of invasion of the host-plant tissues was observed in stem, leaves, or bracts. In numerous cases, however, groups of infective larvae were found lying between the very young leaves surrounding the growing points. Both terminal and lateral growing points were found so infested. Figure 2, A, shows a typical case of such infestation before any sign of a developing inflorescence is evident. Figure 2, B, is a photomicrograph of a longitudinal section through a growing point a day or two older than that of figure 2, A. Sections of nematodes are evident among the leaves. None has penetrated as far as the inflorescence in the center. The method for demonstrating the presence of the nematodes in these figures and the two following was that of killing with an osmic-acid-containing fluid, followed by dehydration and clearing in clove oil, as described elsewhere by the writer (5).

In a collection made one week later, the inflorescence of the plant was evident in the terminal growing point. Examination of some of the floral buds showed nematodes penetrating individual flowers, usually basal ones. Figure 2, C, shows an entire inflorescence just emerging from the leaves at the growing point, with a group of larvae entering a single flower, not through, but between the sepals and petals. Figure 2, D, shows a longitudinal section 12  $\mu$  thick through a flower bud in a similar stage of development. The nematodes lie between the flower parts but in no case within the tissues. While no mechanical injury to flower parts is evident at this stage, there is indication that some feeding on the part of the larvae has occurred. They have definitely increased in size, and the gonad tissues have elongated and become multicellular.

Subsequently, at weekly or bi-weekly intervals throughout the period of growth of the plants, collections were made, and the mutual relations between the infesting nematodes and the developing host plant were studied. At every collection, some of the material taken directly from the plant was killed at once in Flemming's strong killing fluid or in formol-acetic-alcohol and later processed for imbedding in paraffin, and sectioned. Another portion was taken to the laboratory for examination. Figure 3 shows an entire series of collected flowers representing stages of infestation from incipiency to the completely developed gall, with a normal inflorescence for



FIG. 2. A. Nematode infestation of the growing point of *Amsinckia intermedia*. The nematodes lay among the leaves surrounding the growing point, but had not penetrated any of the host tissues. They are stained black at *nem.*  $\times$  about 24. B. Longitudinal section through a growing point at a stage somewhat later than that in A. Sections of nematodes are evident among the leaves. Note the very young inflorescence, not yet reached by any of the nematodes.  $\times$  about 40. C. Single terminal inflorescence with a group of nematodes, *nem.*, entering a single basal flower that was in just the right stage for penetration.  $\times$  about 24. D. Longitudinal section through two adjacent flowers in about the same stage of development as shown in C. Note the sections of nematodes beneath the petals of the flower. No development of gall tissue had occurred.  $\times$  about 40.

comparison. The sectioned material was stained in part with Flemming's triple stain, but mostly with alcoholic haematoxylin adjusted to pH 1.36 according to the method described by Craig and Wilson (1). This method gave excellent results not only in parasite-host differentiation but for the study of nuclear phenomena in host and parasite tissues as well. Figures 2, D, 4, A and B, and 5, A and B, present the sequence of events in the development of the gall.

Once the nematodes are established beneath the petals and in the cavity surrounding the very small pistil, there immediately ensues a remarkable



FIG. 3. Different stages of development of fruit galls in *Amsinckia intermedia*. *a*, a very young inflorescence, with a single flower infested; *b*, a similar inflorescence with all flowers, including an infested one, more advanced; *c*, a double inflorescence, with one flower in each infested; *d*, the single infested flower greatly enlarged by nematode infestation; *e*, a single infested flower, detached, approximately fully developed, as it appears at the end of the growing season; *f*, same as *e*, sectioned longitudinally, to show the inferior infested region, with the slightly darkened infested tissues accentuated with a stain; *g*, a normal double terminal inflorescence, the oldest flowers having attained their maximum size.  $\times$  about 1½.

variation from the normal course of floral development. Stimulated by the parasites, an immediate and pronounced hyperplasia of the floral organs surrounding the nematodes takes place. The highly nutritious food materials that normally go to seed production are now diverted to this abnormal growth, making for the production of a true gall tissue, and this serves as the medium upon which the nematodes feed. They develop quickly into complete sexual maturity and enter at once upon a period of intensive reproduction. The petals, normally only 4 or 5 cells thick, become many times as thick, and they grow in length also. The stamens and anthers soon lose their identity and become a part of the hyperplastic tissue. Within a few days, the nematodes are completely encased, not by

virtue of their penetration into any part of the flower or fruit tissues, but because of the growth of these parts around them. Figure 4, A, shows a single flower in this condition, with the sepals of the calyx still identifiable as such, but with the interior parts of the flower abnormally developed in size and form and completely surrounding the nematodes. These original-invading nematodes are now fully developed, and, as shown by microscopic examination, are just reaching the stage of reproduction. They are



FIG. 4. A. Longitudinal section through a flower about a week older than that shown in figure 2, D. The nematodes, shown at x, (sectioned), have attained approximate adulthood, but no eggs have been deposited. The presence of the nematodes has stimulated rapid increase in host cell growth in all parts of the flower, but particularly the carpillary region.  $\times 37$ . B. Developing fruit gall a few days older than that shown in A. Large numbers of eggs have been deposited, and are to be seen surrounding the sections of the adult nematodes. The hyperplasia that has occurred in the host tissues has completely surrounded the mass of nematodes. C, calyx of the flower; p, abnormal petal; hyp, hyperplastic growth in the carpillary tissues of the flower; nem, adult nematodes; eg, eggs.  $\times 56$ .

gorged with food, and the greatly enlarged sexual organs are reflexed to nearly a quarter of their total length in extreme cases. A typical gall in this stage, about 2 mm. in diameter, contained 7 ♀ and 8 ♂ nematodes. Large numbers of eggs are produced and deposited within the cavity of the gall. The eggs, apparently without any rest period, begin the usual cell divisions; in a few days larvae appear within the cavity in abundance. A gall in the stage represented by figures 4, B, and 5, B, contained 9 ♀ and 8 ♂ adult nematodes, scores of larvae in different stages of development up to about one-half the length of the adults, and hundreds of eggs. The larvae

begin active feeding immediately and quickly grow to maturity. This second generation of mature male and female nematodes, many times the original invading population in number, start reproducing without delay. Just prior to the ripening and hardening of the plant, thousands of eggs are to be found within the cavity of the gall and deep in the smaller cavities extending into the wall.

Meanwhile, the gall has continued to enlarge greatly. Hyperplastic strands of small, sometimes irregularly attenuated cells, are evident in different regions surrounding the locule of the gall in the carpillary region at its base, in the lateral regions, where the gall tissue involves even some of the calyx cells, and even in the apical region. Sometimes uninjured cells of such strands may be recognized immediately adjacent to nematodes free in the locule. In general, however, most of the cells adjacent to the locule are collapsed and show evidence of having been the source of nutriment of the nematodes. The frequency with which cell nuclei undergo division indicates rapid growth of the hyperplastic tissue.

As the plant becomes completely mature, with drying of the stem and cessation of growth in the growing points, the eggs hatch and the larvae feeding upon the remaining nutritive material in the walls of the gall (which becomes permeated by them) attain the secondary larval stage in which they survive through the resting period. At this time adult nematodes are still to be found, as well as many newly hatched larvae. The predominating form, however, is this true resting stage, which is very uniform as to size and stage of development. Examination made in September, 1936, showed that in 3-year-old material, all the older and younger nematodes had perished leaving only this one stage capable of resuming activity.

In the surviving stage sexual differentiation was already evident, as shown by the positions of the 3-celled gonads. The alcoholic haematoxylin stain at pH 1.36, heretofore mentioned, followed by clearing in clove oil, clearly disclosed their position. This position of the gonads was recorded in a random group of 74 larvae in terms of percentage of total length from the anterior end of the nematode. In one group of 36 individuals ( $\text{♀}$ ) the position averaged  $54 \pm 2$  per cent; in the other group of 38 ( $\text{♂}$ ) it averaged  $77 \pm 2$  per cent. Thus, the sex ratio in this random lot was almost exactly 1 to 1.

These nematode-host relationship studies have clearly shown that during the growing season of the plant, the nematodes complete two full life cycles, commencing with the resting stage.

#### ECOLOGICAL SPECIALIZATION IN DITYLENCUS DIPSACI

A comparison of host relations between the very highly specialized strain *Ditylenchus dipsaci* var. *amsinckiae*, on the basis of the studies herein presented, and the somewhat differently specialized strain found in *Hypochaeris radicata* L. reveals the extent to which ecological specialization may occur within this plant parasitic species of nematode.

### Overwintering of the Nematodes

In *Hypochaeris*, the nematodes survive the winter mostly within the hardened but still green and living overwintering leaves of the plant. In *Amsinckia*, winter survival is within the hard, dry, non-living galls produced by the over-grown floral parts of the plant.

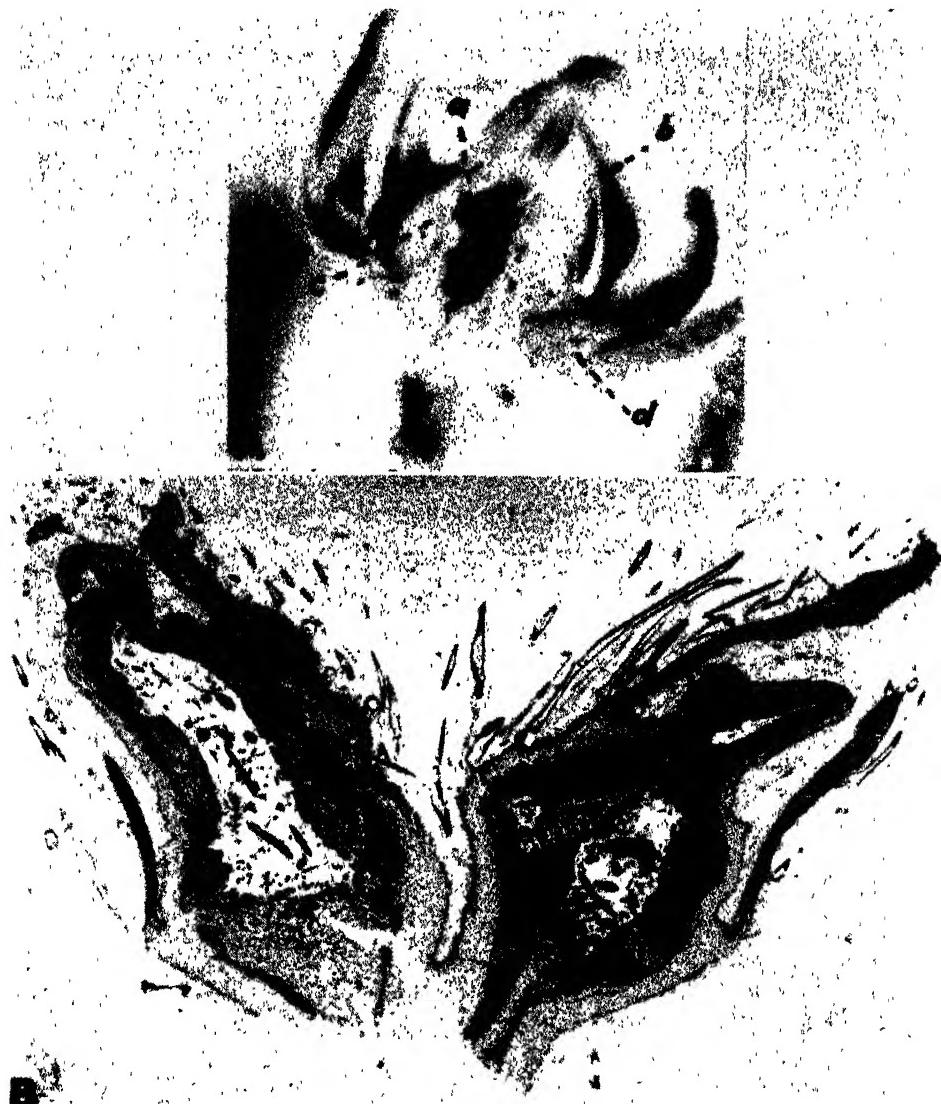


FIG. 5. A. Immature gall dissected partly open to show *a*, the mass of nematodes, adults, larvae, and eggs, in the interior; *b*, the fairly normal appearance of the calyx; *c*, the gall proper, many cells thick, completely surrounding the nematodes, the petals and other flower parts having completely lost their identity; *d*, a normal flower of about the same stage of development. B. Longitudinal section through two adjacent infested flowers, showing in the interior, sections of the original invading nematodes, now adult, and eggs and larvae of a new generation.  $\times 30$ .

### Host-plant Infestation

Invasion of succulent leaf and floral tissues, with resulting distorted growth, is common in *Hypochaeris*. The gall-like swellings are to be found throughout the year. None whatever of this kind of invasion of tissues is to be found in *Amsinckia*.

### Nematode Life Cycles

Within the gall-like leaf swellings in *Hypochaeris*, at any time in the year nematodes are to be found in all stages of development. There are no clearly differentiated generations to be detected, except those connected with the floral parts. In *Amsinckia*, there are 2 distinct generations, and only 2.

### Primary Early Spring Infestation of the Floral Parts

The two plants are alike in that infective larvae are available from the over-wintering source for infestation of the floral parts as soon as they appear. In *Hypochaeris* they are washed or they actually migrate downward to the center of the plant rosette. Here the tender succulent buds appear early. The infective larvae migrate between the inflorescence bracts to their points of attachment, where they penetrate directly into the pith region at the top of the stem and beneath the plate upon which are attached the florets of the inflorescence. This normal path of entrance in *Hypochaeris*



FIG. 6. A. Inflorescence of *Hypochaeris radicata* showing early stage of invasion by the nematode *Ditylenchus dipsaci*. The path of entrance is not through the individual flower, as in *Amsinckia*, but between the bracts of the inflorescence, and thence by actual penetration of the tissues, into the pith region beneath the inflorescence. Nem., nematodes.  $\times$  about 25. B. A later stage of infestation in *Hypochaeris radicata*. The cavity in this case is produced entirely by the mechanical activities of the nematodes, and not by any growth reaction on the part of the host plant.  $\times$  about 25.

*ris* is illustrated in figure 6, A. In this region of primary infection the larvae migrate freely, feed upon and destroy the cell contents, mature promptly, and reproduce. The mature adult nematode stage is shown in figure 6, B. The young migrate upward while feeding upon the parenchyma cells of the stem. This new generation of infective larvae, at just the right stage of development of the florets, penetrates the seed coat at its base and becomes established in a temporary resting stage next to the uninjured seed. With *Amsinckia*, as shown previously in this paper, infestation of the flower is by external migration among the leaves of the growing points and between the petals of the developing flower, eventually becoming localized within the flower. This is followed by hyperplasia of the floral parts completely enclosing the nematodes.

*Modes of Dissemination.* Herein lies one of the most striking differences between these 2 highly specialized strains of *Ditylenchus dipsaci*. In *Hypochaeris*, dissemination takes place by means of the wind-blown pappus-bearing seeds. Details of proof of this have been presented heretofore (2, 4). This results in widespread distribution of the nematosis produced by this strain. It occurs more or less uninterruptedly from Puget Sound to San Francisco Bay and in many parts of Europe and probably Asia. With *Amsinckia*, dissemination in nature depends entirely upon the survivors of the nematodes from fallen galls. The range of *Amsinckia* nematosis is correspondingly limited to small loci of infestation.

#### SUMMARY

The plant infesting nematode, *Ditylenchus dipsaci* var. *amsinckiae*, in its manner of producing galls in its host plant *Amsinckia intermedia* Fish. and Mey., displays a remarkable specialization in the selection of host tissues invaded. In this respect it differs strikingly from other strains of *Ditylenchus dipsaci*. Primary infestation of the plant is among the leaves surrounding the growing point, whence it enters the developing flowers. Entrance is gained not by direct tissue penetration, but by migration between the floral parts to the space adjacent to the ovary. Here their presence stimulates active hyperplastic growth of the floral parts, resulting in complete enclosure of the nematodes and the formation of a gall much larger than the normal fruit. Within this developing gall the nematodes feed and pass through two complete life cycles, eventuating in a tremendous population (as many as 40,000 in a single gall) of sexually differentiated resting-stage larvae. The firm leathery gall, fallen to the ground, serves as a protection to the nematodes until the advent of the next growing season, when they are available to initiate new infections in the seedling plants. There being no provision for widespread dissemination of the parasite, infestations of the plant are distinctly localized. The comparison is made between this manifestation of highly specialized host-parasite ecological relationship, and that occurring with the same species of nematode in *Hypochaeris radicata*. In the latter, direct tissue penetration

occurs, with development of gall-like swellings in leaves and floral parts, and in addition the seed capsules are penetrated, and the nematodes are disseminated by means of the wind-blown pappus-bearing seed. This results in widespread distribution of the Hypochoeris strain of the nematode.

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BROWN BLIGHT OF LETTUCE

I V A N C . J A G G E R <sup>1,2</sup>

(Accepted for publication July 25, 1939)

INTRODUCTION

This paper is a preliminary report on the brown blight of lettuce, recording investigations carried out in the Imperial Valley of California from 1922 to 1927. In the fall of 1922 the writer began the investigation of this new disease of lettuce in the Imperial Valley of California. At that time lettuce production in this section was relatively a new industry, and the disease was threatening to seriously curtail production.

Brown blight was apparently unknown until lettuce culture became important in the Imperial Valley. In a few years it increased rapidly and

<sup>1</sup> Late Senior Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>2</sup> The manuscript of this article, as prepared by Mr. Jagger, was apparently never considered by him to be a complete or conclusive treatment of the subject. It was found among his records after his death and is published essentially as originally prepared several years ago because it presents the only known detailed description of the disease, together with other observations of historical importance and technical interest. These circumstances must be kept in mind when judging the article in the light of present knowledge.

was called to the attention of State and Government pathologists by local growers in 1917 or 1918. D. G. Milbrath<sup>3</sup> made observations on the trouble in the field and recognized it to be an undescribed disease, but did not publish on it. Since 1922 several popular accounts of the disease and its investigation have appeared in local newspapers and various agricultural publications.

#### COMMON NAME

When investigations were begun in 1922 various names were locally applied to the disease, but the general term "sick" or "diseased" lettuce was most frequently heard. In conferring with Imperial Valley people the name "brown blight" was hit on, and was proposed in an article published in local newspapers in February, 1924. It is now quite generally used.

#### DISTRIBUTION

So far as known the disease occurs only in California and Arizona. In California it is very destructive in the Imperial Valley. Occasional possibly affected plants have been found in Orange, Los Angeles, and Salinas counties. If it does occur in these counties, it is increasing much more slowly than in the Imperial Valley and is of no economic importance at present. In 1925 it was noted in Arizona in the vicinity of Yuma and Phoenix, threatening serious injury in the former section, but apparently developing less rapidly in the latter.

#### HOST PLANTS

Brown blight is known to attack only lettuce (*Lactuca sativa* L.). A large number of crops and many species of weeds have been observed making normal growth on soil so severely infested that 75 to 100 per cent of the comparable adjacent or preceding crop of lettuce was destroyed. Crops observed include garden pea, cowpea, cotton, cantaloupe, carrot, alfalfa, barley, grain sorghums, red table beet, and endive (*Cichorium endivia* L.).

#### DESCRIPTION

Seedlings are never affected until they have developed 4 or 5 leaves each, but, thereafter, plants are attacked in all stages of growth. On severely infested land, seedlings nearly always appear entirely normal until after thinning. Symptoms depend on the stage of growth at which the plants are attacked.

When attacked while small the first symptom is the appearance of small, light yellow, discolored spots in the young expanding leaves at the centers of the plants (Fig. 1). The yellow spots are very distinctive and seem to be an unmistakable symptom of brown blight. At first the spots are almost indistinguishable in bright sunlight, but can be readily seen on shading the plants. As the leaves expand the spots enlarge somewhat and the leaf areas between become sickly yellowish green. These and all subsequent leaves

<sup>3</sup> Plant pathologist; State Department of Agriculture, Sacramento, Calif.

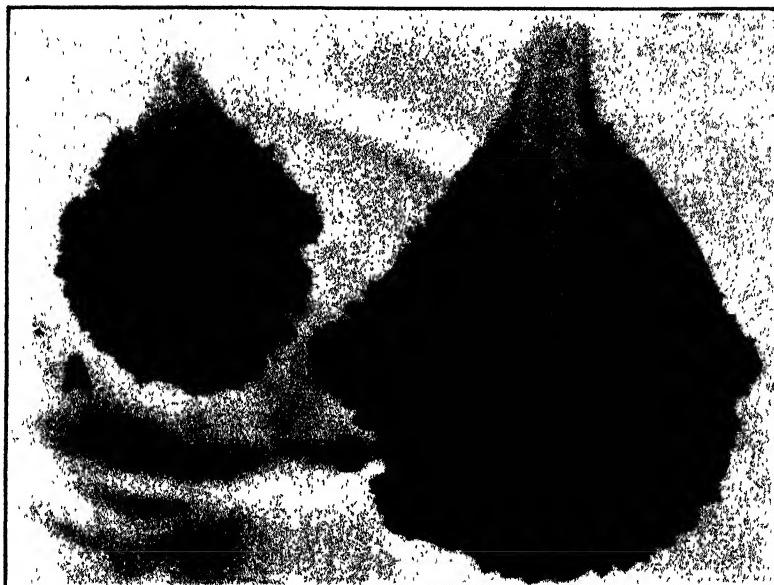


FIG. 1. A. Lettuce leaf, showing light yellow specks, the first symptom of brown blight. B. Healthy leaf.

are much reduced in size and tend to lie flat on the ground, producing small, much stunted, rosette-like, discolored plants, which are very conspicuous in the field (Fig. 2). Sometimes the older leaves at the base, fully expanded



FIG. 2. Lettuce field, showing brown-blight infested area in center foreground with diseased discolored, much stunted plants in contrast with healthy plants in the lower corners and background.

when the plant was attacked, retain a healthy appearance for some time, contrasting strongly with the younger, stunted, discolored leaves above. Finally the stunted plants show a gradual browning and dying of the leaves, progressing upwards from the bases, and many plants are entirely dead before harvest.

Plants that are attacked after the heads have begun to form first show dead, brown, irregular, disconnected, more or less sunken blotches and streaks in the leaves (Fig. 3). The brown dead tissues are firm and dry

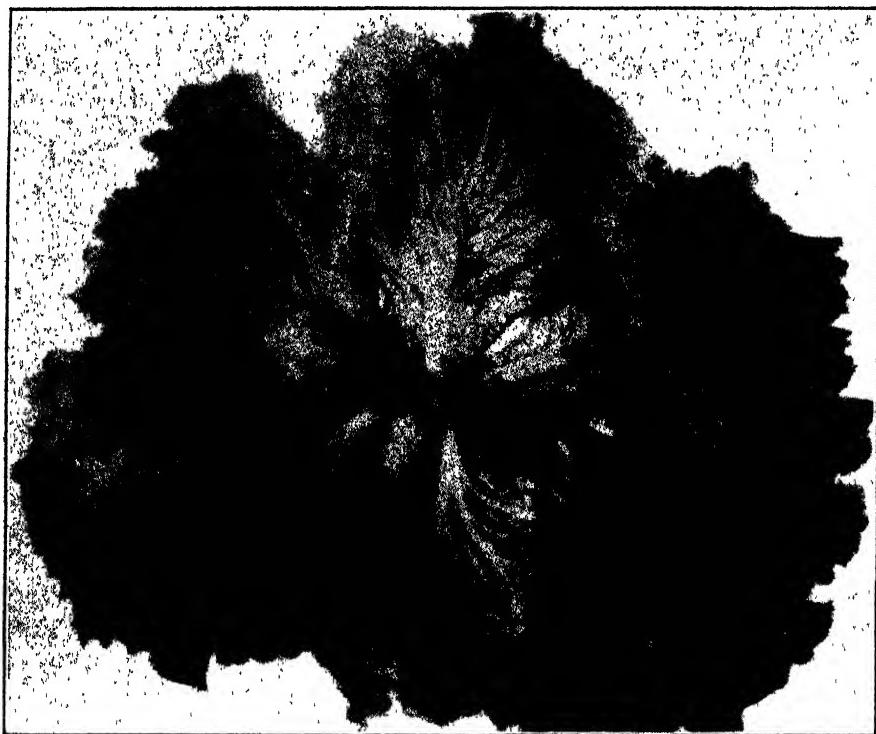


FIG. 3. Head of lettuce attacked by brown blight, with loose wrapper leaves on near side broken back, showing brown dead irregular blotches and streaks in several leaves.

unless invaded by secondary soft-rot organisms. The dead areas are variable in size, shape, and distribution. They usually are associated with the midribs and larger veins of the leaves, but may occur between veins or along the smaller veins. The dead brown streaks sometimes extend into the vascular tissues of the stem for a short distance, but are frequently confined to the leaves. In the loose wrapper leaves and the outer leaves of the head the lesions occur largely in the basal portions, but frequently extend well towards the tips in the covered leaves of the head. A large percentage of the leaves, or only an occasional leaf, may show lesions. A few of the outer leaves of the head usually show the most pronounced lesions, which become less prominent toward the center and are always absent from several layers of the smaller heart leaves at the center of the head. While these lesions are

developing, the general color of the plant gradually changes from dark green to a sickly yellowish green, the leaves and head become more or less flabby, growth all but ceases, and finally there may be a gradual browning and dying of the outer leaves.

There are, of course, all combinations of the two sets of symptoms in plants attacked in intermediate stages of growth. Additional symptoms, which seem to be of a secondary nature, frequently occur. Heads attacked when nearly mature sometimes show a dark brown, moist, slippery condition of the small heart leaves. In early stages of the disease the roots appear to be normal; but in advanced cases, when the plants are stunted and gradually dying, they show much discoloration and dying back from the tips. Affected plants are more readily injured by frost than healthy ones. Organisms, which cause soft rot or "slime," obtain a foothold in the dead brown lesions of affected plants when conditions are favorable.

In lettuce fields there may be only an occasional diseased plant, or there may be irregular areas from a few feet to several rods in diameter, within which essentially all plants are diseased. The more or less isolated infested areas where the plants are stunted and dying are very noticeable in moderately infested fields. In severe cases a high percentage of plants over whole fields may be affected. A considerable percentage of plants, destined to be affected, usually are attacked while small. An occasional crop, however, may show only a few diseased plants during the early part of the growing season and be severely attacked when nearly ready to harvest.

All plants attacked before maturity are either dead or stunted, discolored, and useless at harvest time. Occasional plants, attacked when almost ready to harvest, appear to be normal on superficial examination and are not detected in harvesting and packing. Heads showing the characteristic dead, brown, irregular streaks and blotches on removing a few outer leaves are sometimes found on the market.

#### BROWN BLIGHT IS SOIL-BORNE

The distribution and spread of the disease in fields is characteristic of a soil-borne trouble. Usually there is less than 1 per cent of diseased plants where lettuce is grown for the first time, but with continued cropping there is a rapid increase each succeeding year until 75 per cent or more of the plants may be diseased in the third or fourth crop of lettuce. Numerous cases have been observed where the disease was limited to a few isolated areas in a field, and in the next crop the disease was centered in the same areas, which were, however, markedly larger. Of two fields separated only by a ditch or a fence, one often shows a high percentage of diseased plants, usually where previous crops of lettuce have been grown, and the other, only a few feet distant, an unimportant percentage.

In numerous experiments pots and boxes of soil from severely diseased fields or areas produced lettuce that showed nearly 100 per cent characteristic brown blight, while comparable pots and boxes containing soil from

healthy fields or areas or from uninfested regions produced disease-free lettuce. The disease developed fully as well in the coastal climate at Chula Vista, San Diego County, Calif., as in the decidedly different Imperial Valley, and all experiments of this nature were carried out there. In most of the experiments plants were grown in 6-inch clay pots, usually 4 plants in a pot. Plants were somewhat crowded and undersized in later stages of growth, but grew sufficiently well to readily show brown-blight symptoms. When plants became an abnormally yellowish color, indicating lack of nutrients in the limited soil, they were quickly brought back to normal by watering for a few days with water containing 0.1 per cent of  $\text{NH}_4\text{NO}_3$  and 0.05 per cent of  $\text{K}_2\text{HP}_2\text{O}_5$ . There were never any indications of the disease being carried from diseased to healthy pots by insects or by soil splashed in watering, although diseased and healthy pots were adjacent in many cases.

#### NOT ALKALI POISONING

It has been repeatedly suggested that the trouble might be because of an excess of alkali salts in the soil, a supposition based apparently on the facts that brown blight, like alkali poisoning, occurs first in isolated areas that increase in size from year to year, and that alkali occurs in all known brown-blight-infested regions. Since germinating seed and small seedlings never are affected by brown blight, no other crops or weeds seem to be susceptible, and since the usual symptoms of alkali poisoning are lacking, alkali poisoning seems precluded. In order to test definitely the alkali-poisoning theory, sufficient distilled water was leached through pots of infested soil to reduce the content of soluble salts to a very small percentage of the original, as determined by the electrical-bridge method (1). There was fully as high a percentage of brown-blight-diseased plants in the leached pots as in untreated check pots.

#### SOIL STERILIZATION DESTROYS CAUSAL AGENT

The close association of the disease with the soil suggests an organism parasitic on the roots. In order to throw further light on this possibility pots were filled with infested soil; a portion was sterilized by steaming 1 hour under 10 to 15 pounds' pressure; part was sterilized by drenching with 40 per cent formaldehyde, diluted 1 part to 20 parts of water; part was given no treatment. Lettuce was grown in all under like conditions. The combined results from 3 experiments were 58 healthy and 0 diseased plants in 15 steam-sterilized pots; 60 healthy and 0 diseased plants in 15 formaldehyde-sterilized pots; and 2 healthy and 58 brown-blight-diseased plants in 15 untreated check pots.

#### ORGANISMS

Attempts were made to isolate an organism from the brown dead lesions of older plants with no indications of success. Later, as a result of the soil experiments, the search was transferred to the roots. As already stated roots appear to be entirely normal in early stages of the disease but micro-

scopic and cultural examination shows a considerable number of fungi associated with them. A large percentage of the roots of lettuce and many other crops and weeds in Imperial Valley are heavily invaded by an organism that is indistinguishable from the mycorrhizal fungus studied by Jones (5). This is fully as abundant in healthy as in diseased plants and apparently has no connection with brown blight. Several species of *Pythium*-like fungi were isolated from the roots of diseased plants and tested as possible causes of the disease, although studies to the present time indicate that the same species are associated also with the roots of healthy plants.

Finally, a fungus, which seems to be *Asterocystis radicis* de Wildeman, has been repeatedly found in abundance in the epidermal cells and root hairs of diseased plants, and has been found only rarely and in limited amounts in healthy plants. This fungus is widespread in Europe, where it has been recorded in the roots of many species of plants by de Wildeman (8), Marchal (6), Ducomet (2), Fron and Gaillat (3), and others. In most species the fungus seemed to cause no appreciable injury to the hosts. Diseases of flax, oats, and grasses, however, are attributed to it in the last three references, although entirely conclusive proof of a causal relation was obtained in no case. As in the European investigations, inability to grow the fungus in pure culture has made it difficult to determine whether it causes disease in lettuce. At present, *Asterocystis radicis* can be mentioned only as a possible cause of brown blight of lettuce.

#### SYMPTOMS SUGGEST A MOSAIC

Brown-blight symptoms are suggestive of a transmissible mosaic disease, although the common mosaic disease of lettuce (4) is very distinct from brown blight. Unsuccessful attempts were made to transmit brown blight by the usual experimental methods of transferring insects and that of injecting juice from diseased plants, but that line of investigation was abandoned on discovering the soil relations of the disease. Further studies are now indicated, since brown blight is strikingly similar in many respects to the disease of wheat and rye, shown by McKinney (7) to be a transmissible virosis, the causal agent of which is associated with the soil. There are no indications that the two diseases are identical, but they might well be of similar nature.

#### CONTROL

##### Crop Rotation

On account of lettuce culture being a comparatively new industry in infested regions, only limited data on the effect of crop rotation have been obtainable. It has, however, been conclusively demonstrated by the experiences of several growers that growing alfalfa or other crops besides lettuce for 3 or 4 years causes little or no reduction of infestation. In fact, general observations have in several cases suggested an increase in soil infestation during 1 to 4 years of crops other than lettuce, but the increase was much slower than with lettuce.

### Avoiding Infested Land

In the past, sufficient acreage suited to lettuce has been available, so that serious losses have been largely avoided by constantly shifting to land where lettuce has never before been grown. In the Imperial Valley diseased plants can be found in nearly all fields, but there is usually less than 1 per cent where lettuce is being grown for the first time. Occasionally, there is sufficient disease in first-year lettuce to cause appreciable reductions in yield. Second-year lettuce usually makes a satisfactory crop; but, in general, there is a higher percentage of brown-blighted plants, and cases of economic losses are more numerous. Usually, third-year lettuce is so seriously injured that it does not make a profitable crop, although occasional third-year and even fourth-year crops show only limited disease. In the Imperial Valley it has become customary to grow two crops of lettuce on land, and then to pass on to land where lettuce has never been grown before.

Careful counts were made in a first-year lettuce field of 10 acres that showed more brown blight than usual, and the next season similar counts were made in second-year lettuce on the same field to obtain information on rate of increase. A total of 4,000 plants in the first-year crop showed 3.7 per cent of brown blight, and the same number in the second-year crop showed 20.3 per cent of brown blight. In another case, which is perhaps more typical, there were only 2 (0.05 per cent) diseased plants among 4,000 in first-year lettuce and 101 (2.5 per cent) among 4,000 in the second-year crop. In a second-year field of 20 acres, estimates as well as yields indicated that 75 per cent of the plants were affected with brown blight. Many third-year fields have been observed where counts and estimates have indicated from 15 per cent to as high as 90 per cent of diseased plants.

These data, as well as general observations, indicate that on land where a crop shows over 1 per cent of disease it is in general not advisable to grow another crop of lettuce. So far as is known at present it is not safe to grow lettuce on land where this crop has been grown in previous years and followed by other crops for several years. Occasionally crops are injured by brown blight in spite of every precaution to avoid infested land, since land that has never grown lettuce is sometimes infested, and since the previous cropping may not be accurately known.

### RESISTANT VARIETIES

In 1923 over 100 varieties of lettuce were grown on infested land. As the number of plants was limited and the field showed areas of light infestation the results were not conclusive. Most of the varieties showed some brown blight, but the two varieties, Big Boston and Chavigne, were disease-free. Several hundred plants of these two varieties were grown on severely infested soil in 1924 and again in 1925 and made entirely normal growth, with no indications of brown blight, whereas check plants of the New York variety showed a high percentage of disease. It thus seems conclusively demonstrated that both varieties are highly resistant, if not entirely im-

mune. Several hundred second-generation hybrids from a cross between Chavigne and New York were grown on severely infested soil in 1924, and in 1925 gave healthy and diseased plants in ratios of approximately 3 to 1, thus indicating that resistance to brown blight probably behaves as a Mendelian dominant character. The obtaining of brown-blight-resistant strains of the New York type by selection from the hybrids seems possible, although progress in that direction is now overshadowed by the resistant selections from the New York variety.

The brown-blight-resistant strains, obtained by selecting within the variety New York, seem to show no resistance to the obscure and so far unimportant pathological condition designated as "big vein." Big Boston and Chavigne, however, seem to be resistant to or immune from both diseases. Commercially, these varieties are of little or no value in infested regions, but hybridizing with New York offers possibilities of obtaining New York types that are resistant to both brown blight and "big vein."

#### Resistant Strains of Variety New York

In 1924 a field of badly diseased lettuce was found where many of the plants showed marked indications of resistance (Fig. 4). One hundred



FIG. 4. Parent plant of brown-blight-resistant lettuce, New York Imperial No. 2, as found in severely diseased field with all surrounding plants diseased or dead, Imperial Valley, 1924.

promising plants were selected and seeded. As lettuce is largely self-pollinated, no precautions against cross-pollination were necessary. From each of the 100 lots of seed 25 to 100 plants were grown on infested soil in 1925.

From the time brown blight commenced to develop it was evident throughout the season that a surprisingly large number of the selected strains possessed a pronounced degree of resistance. Commercial seed planted as a check showed a high percentage of brown blight in all parts of the trial area, 85.5 per cent of a total of 2,362 check plants being diseased. In 55 of the selected strains there were no diseased plants throughout the season. It was hoped that some of these might prove to be entirely immune, but, as recorded below, all strains so far tested on a large scale on heavily infested soil have developed a small percentage of disease. Of the remaining 45 strains nearly all showed indications of resistance.

The field where the above selections were made apparently was planted with different commercial seed from that commonly used, and finding the field seems to have been a matter of good fortune, for it resulted in resistant strains being isolated and established almost immediately. The lettuce in this field seemed typical of the variety New York, although there were more sports and off-type plants than in most fields. Search for resistant plants had been made in many fields without finding anything of promise until this field was visited. At the time of making selections in this field, 35 less promising plants were selected and seeded in 3 other fields where there was the usual absence of plants showing definite indications of resistance. When these 35 lots of seed were tested in 1925, there was no indication of resistance in 30, while the other 5 lots, though somewhat resistant, were otherwise worthless.

Most of the selections made in the promising field were of the New York



FIG. 5. Experimental planting, 1926. In center, a double row from non-resistant commercial seed with over 90 per cent of the plants diseased and dying. On each side, a 2-acre block of selected resistant strains numbers 2 and 3 with less than 0.5 per cent plants diseased by brown blight.

type, although some sports and off-types were included. In the 1925 progenies some strains were extremely variable; others were unusually uniform. Of the highly resistant, reasonably uniform strains, several that differed appreciably from each other in minor characters were very similar to the New York variety. As soon as the 1925 plantings reached a stage where the commercial value of the different strains could be judged, the 3 most promising were chosen for growing sufficient seed for commercial trial in 1926. The remaining seed of these strains, obtained from the 3 parent plants selected and seeded in 1924, was then planted in a favorable locality, and several pounds of seed of each strain were harvested in the autumn of 1925.

In 1926, 2 acres of each of the 3 resistant strains were grown in a severely infested experimental field, and smaller areas in several commercial fields where there was only a limited amount of disease. All 3 strains showed a high degree of resistance (Fig. 5). All developed a small and commercially unimportant percentage of brown blight on severely infested soil, and also a small percentage of the obscure pathological condition designated as "big

TABLE 1.—Percentages of diseased plants in trial plantings of selected brown-blight-resistant strains of the New York variety of lettuce and of commercial seed of the same variety in adjacent check rows, Imperial Valley, California, 1926

Row No.	Varieties and strains	Counts made 5 to 6 weeks before harvest		Counts made a few days before harvest	
		Total plants	Brown-blight-diseased plants	Total plants	Brown-blight-diseased plants
		Number	Per cent	Number	Per cent
<i>Severely diseased experimental field</i>					
16	Resistant strain No. 1	1263	0.08	1251	0.08
17	Commercial .....	1022	69.77	916	92.47
42	Commercial .....	1063	78.27	901	90.46
43	Resistant strain No. 2	1182	0.17	1157	0.35
82	Resistant strain No. 2	1210	0.25	1176	0.33
83	Commercial .....	1133	76.10	912	94.80
123	Resistant strain No. 3	1090	0.55	1169	0.19
124	Commercial .....	1311	69.03	1110	92.07
125	Resistant strain No. 1	1191	0.25	1183	0.25
<i>A commercial field—2nd-year lettuce</i>					
85	Resistant strain No. 3			1350	0.07
86	Commercial .....			1427	3.64
93	Resistant strain No. 2			1256	0.00
94	Commercial .....			1433	3.63
97	Resistant strain No. 1			1411	0.00
98	Commercial .....			836	5.03
<i>Another commercial field—2nd-year lettuce</i>					
61	Commercial .....	958	5.50		
62	Resistant strain No. 1	1092	0.00		
71	Commercial .....	1040	0.58		
72	Resistant strain No. 2	1081	0.00		
81	Commercial .....	958	0.63		
82	Resistant strain No. 3	867	0.00		

vein." The strains were very uniform in type but quite distinct from each other. Plants of all three types occur in nearly all commercial fields, but in the better commercial fields most of the plants are very similar to those of Strain No. 2. Strains 1 and 3 are of very doubtful commercial value, since they frequently fail to head as well as plants from commercial seed. Strain No. 2 gives promise of being a very satisfactory commercial lettuce for the Imperial Valley on either infested or disease-free soil. It has not been tested in other sections. The following table (Table 1) gives percentages of diseased plants in the 3 strains and in check plantings of high grade non-resistant commercial seed.

The promising strain, No. 2, has been given the name Imperial No. 2. The demand for seed of this strain was becoming so great that it seemed necessary to turn the growing of seed into commercial channels. Stock seed, identical with that used in the 1926 trials, was turned over to two competing seed growers, who supply a considerable proportion of the seed used in Imperial Valley for growing seed crops during the summer of 1926.

#### SUMMARY

Brown blight, a new disease of lettuce, is causing increasing losses in the Imperial Valley of California and in parts of Arizona. It has been shown to be soil-borne. A root parasite is suspected as the cause, although the striking similarity of brown blight to the soil-borne mosaic disease of wheat suggests the possibility of a similar nature. Crop rotation gives little promise of control. Heretofore, losses have been largely avoided by growing usually two crops of lettuce then shifting to land where lettuce has never been grown. Certain varieties are highly resistant or entirely immune, but are commercially useless in the infested regions. Through selection from the almost exclusively grown, very susceptible variety New York, a highly resistant strain has been obtained which is coming into commercial use under the name Imperial No. 2.

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# DIURNAL CYCLE OF SPORE MATURATION IN CERTAIN POWDERY MILDEWS<sup>1</sup>

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## INTRODUCTION

Massee's<sup>4</sup> observations of *Sphaerotheca humuli* on vegetable marrow indicated that dissemination of conidia occurs principally at night. Hammarlund<sup>5</sup> made extensive and detailed studies of the process of spore maturation in several powdery mildews. He reported that the number of conidia formed per day per conidiophore of *Erysiphe communis* (*E. polygoni*) varied from 1 to 6, and that the conidia were forcibly discharged a distance of 10 to 20 conidial lengths, but he reported no diurnal periodicity. Active discharge also was found by him in *Sphaerotheca pannosa* and other conidial chain forming Erysiphaceae. Yarwood<sup>6</sup> reported that *E. polygoni* on clover showed a marked diurnal cycle in several aspects of its development, and that each conidiophore formed 1 conidium per day, which was passively liberated about midday.

In the conidial chain-forming powdery mildews *Erysiphe cichoracearum* and *Sphaerotheca* spp. Blumer<sup>7</sup> has reported that the basal cell is the conidial-mother cell (generative cell). Foëx's<sup>8</sup> drawings of the evolution of the conidiophore of *Sphaerotheca humuli*, indicate division of the basal cell and also division of the cell just above it. In the non-chain-forming powdery mildew *E. polygoni*, the generative cell is separated from the sporiferous hypha by a stipe cell.<sup>6, 7</sup>

## MATERIALS AND METHODS

In this paper are reported studies on the diurnal cycle of morphological development of the conidiophores of *Erysiphe cichoracearum* DC. from *Helianthus annuus* L., from *Cucumis sativus* L. and from *Aster* sp.; of *Podosphaera leucotricha* (E. and E.) Salm. from *Pyrus malus* L.; of *Sphaerotheca pannosa* (Wallr.) Lév. from *Rosa* sp.; of *Erysiphe polygoni* DC.

<sup>1</sup> The advice and assistance of Dr. C. E. Yarwood, Division of Plant Pathology, University of California, Berkeley, California, in the preparation of this paper is gratefully acknowledged.

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<sup>3</sup> The assistance of non-technical employees of the Federal Works Progress Administration is acknowledged.

<sup>4</sup> Massee, G. E. On the origin of parasitism in fungi. Roy. Soc. [London] Phil. Trans. 197B: 7-24. 1905.

<sup>5</sup> Hammarlund, C. Zur Genetik, Biologie, und Physiologie einiger Erysiphaceen. Hereditas 6: 1-126. 1925.

<sup>6</sup> Yarwood, C. E. The diurnal cycle of the powdery mildew *Erysiphe polygoni*. Jour. Agr. Res. [U. S.] 52: 645-657. 1936.

<sup>7</sup> Blumer, S. Die Erysiphaceen Mitteleuropas mit besonderer Berücksichtigung der Schweiz. 483 pp. Gebr. Fretz A. G. Zürich. 1933.

<sup>8</sup> Foëx, E. E. Evolution du conidiophore de *Sphaerotheca humuli*. Bull. Soc. Mycol. France 29: 251-252. 1913.

from *Phaseolus vulgaris* L., and of *Oidium euonymi-japonici* (Arcang.) Sacc. from *Euonymous japonicus* L.f.

Leaves recently infected with powdery mildew were gathered every 2 hours over 24-hour periods at Berkeley, California. By folding the leaf, or cutting a narrow strip of the lamina, a row of erect conidiophores was obtained that was examined microscopically with a high power objective. For sunflower, cucumber, bean, and apple powdery mildews material was obtained from greenhouse plants; material for other mildews was from outdoors.

The conidiophores studied were of 2 types reported by Blumer.<sup>9</sup> The *E. polygoni* (*E. communis* Wallr.) type are relatively simple in structure and consist of a basal cell, a generative cell, and one or two maturing conidia, depending on the time of day observed. This type of conidiophore will be referred to herein as a non-chain-forming type. Conidiophores of the *E. cichoracearum* type consist of a more or less cylindrical stipe or basal cell, 1 to 3 cylindrical cells above the basal cell, and a chain of from 2 to 8 or more swollen conidia. This type of conidiophore will be referred to herein as a conidial chain-forming type (Fig. 1). Because of the vari-

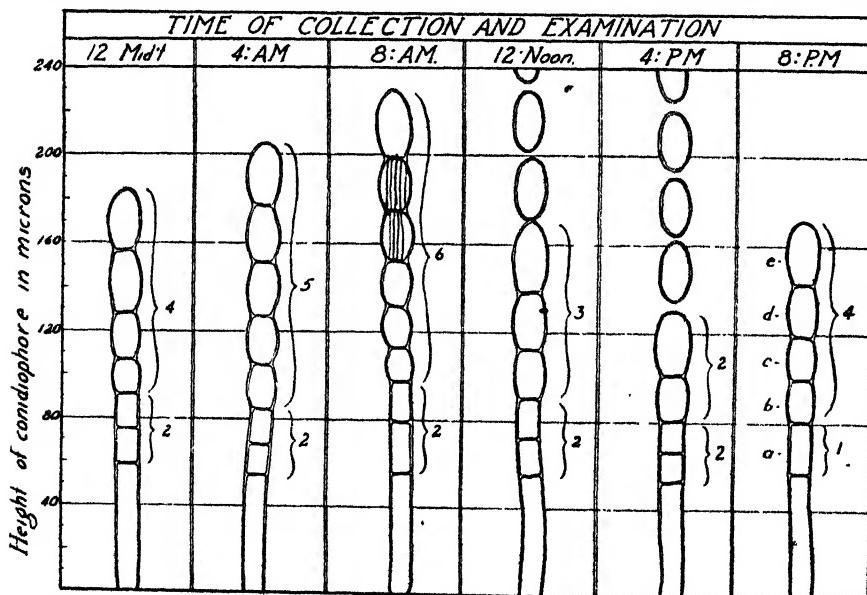


FIG. 1. A diagram of the diurnal cycle of maturation and abstraction of conidia in the cucumber powdery mildew, *Erysiphe cichoracearum*. The line between swollen and cylindrical cells was drawn where there was the greatest difference between their respective shapes.

able number of cylindrical cells above the basal cell, and the variable number of swollen cells above the cylindrical cells, a numerical method of designating the various stages in the development of the conidiophore was necessary for recording the observations. The basal cell was not considered in the final numerical records because it was relatively constant in length

<sup>9</sup> See footnote 7.

throughout the diurnal cycle. The cells distal to the basal cell were classified according to a binumerical scheme: the first member of the binumeral, e.g. 3+2 (Fig. 1 at 12: Noon), refers to the number of swollen cells in a chain of conidia, and the second member refers to the number of cylindrical cells above the basal cell. The number of swollen cells of a conidiophore may amount to 10 or more, e.g. *S. pannosa* on rose, but the number of cylindrical cells above the basal cell is rarely more than 3. Abstricted but still adhering spores (Fig. 1 at 12: Noon) are disregarded.

The above observations were made during the summer months as follows: *Erysiphe cichoracearum* on sunflower, *Sphaerotheca pannosa* on rose, *E. cichoracearum* on aster, during the middle of July, 1937; and *E. cichoracearum* on cucumber, *S. pannosa* on rose, *Podosphaera leucotricha* on apple, *E. polygoni* on bean and *Oidium euonymi-japonici* on *Euonymous* in June and July, 1938.

To supplement the information secured by the direct observation of living conidiophores, the diurnal cycle of conidiophore development was followed by determining the number of spores liberated from infected leaves at periodic intervals, and by observations of the nuclei in stained conidiophores.

To determine spore liberation, young naturally infected sunflower leaves, excised and kept alive on 5 per cent sucrose solution, in Petri dishes and in a well lighted room, were removed periodically and snapped vigorously into a pint can, at the bottom of which a clean slide had been placed. It is believed that in the main, only conidia that have been abstricted will be dislodged by snapping. The number of conidia thus caught was determined microscopically.

To determine the nuclear condition in the conidiophore, primary leaves of sunflower infected with *Erysiphe cichoracearum* were collected periodically from greenhouse-grown plants in December, 1938, and cut into narrow strips bearing rows of erect conidiophores. These conidiophore-bearing strips were then fixed in formalin-alcohol-acetic acid, stained with acid fuchsin in water and examined microscopically.

## RESULTS

### Diurnal Cycle of Sporulation as Shown by Microscopic Examination

Examination of the stages of conidiophore development at 2-hour intervals during 24-hour periods reveals a definite diurnal cycle of maturation and abstraction of conidia in the chain-forming powdery mildews, *E. cichoracearum* (Fig. 1, 2, Table 1), *Sphaerotheca pannosa* (Fig. 2, Table 2), and *Podosphaera leucotricha* (Fig. 2). Diurnal cycles of maturation and abstraction of conidia also were observed in the non-chain-forming mildews, *Erysiphe polygoni* (Table 3) and *Oidium euonymi-japonici* (Table 4). With reference to *E. cichoracearum* on cucumber (Fig. 1, Table 1) the modal binumerical type (heavy type) was 6+2 at 6 to 8 a.m.; by 2 to 4 p.m. it had decreased to 2+2, due to the rapid abstraction of conidia during

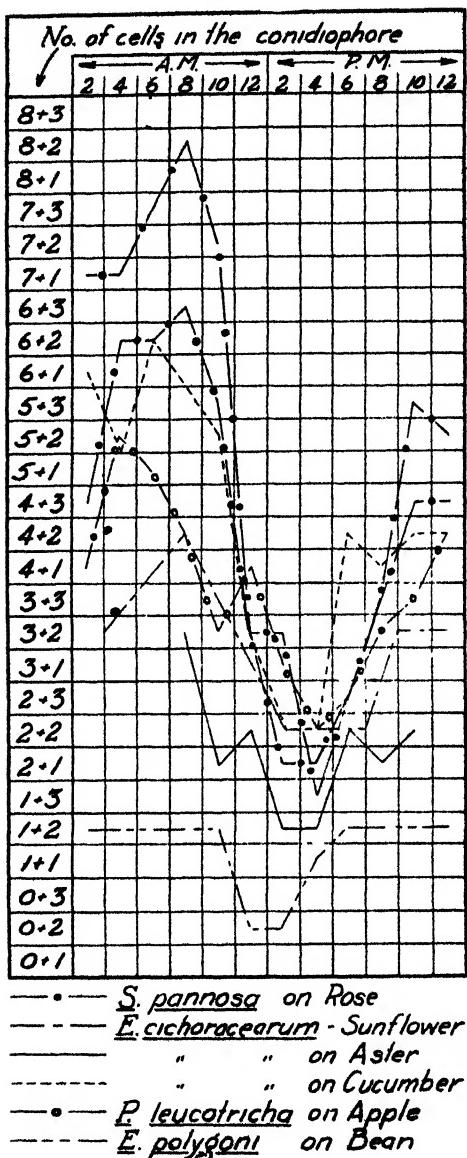


FIG. 2. A graphical summary of the diurnal cycles observed for conidial chain-forming powdery mildews and a comparison between the diurnal cycles of chain-forming powdery mildews and a non-chain former, *Erysiphe polygoni*. *Oidium Euonymi-japonici* is not represented but is very similar to *E. polygoni* in sporulation diurnal cycle. Curves are plotted from the modal binumerical type for each mildew at each period of observation. There is variation among the chain-formers, but none approach the simplicity and regularity of the cycle of the non-chain former, *E. polygoni*.

the elapsed time. From 4 p.m. to 6 a.m. the modal binumerical type rose again to 6 + 2 due to the formation of the succeeding crop of conidia. At the low phase of the cycle, e.g., 2 p.m. to 4 p.m. (Fig. 1 and Table 1) the modal binumerical type was clearly defined, while at the high phase of the

cycle, e.g., 4 a.m. to 10 a.m., the modal type was less clearly defined owing to the presence of young conidiophores that were sporulating for the first time, and old conidiophores whose activity was waning. Hammarlund<sup>10</sup> reports the life of a conidiophore as 4 to 6 days for a number of Erysiphaceae. Similar diurnal cycles were observed for powdery mildews of rose, aster, sunflower, and apple (Fig. 2, Table 2).

TABLE 1.—Diurnal cycle of maturation and abstraction of conidia of the cucumber powdery mildew, *Erysiphe cichoracearum*. Each number in the body of the table represents the number of conidiophores of the binumerical type indicated that were observed at the time indicated. The modal binumerical type for each period of observation is printed in heavy type. The diurnal cycle of conidiophore development is graphically illustrated by the vertical fluctuation in bold-face numbers from 2 a.m. to 12 p.m.

No. of cells in conidio- phore exclu- sive of basal cell	Diurnal cycle of maturation and abstraction of conidia											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
8+3												
8+2			4									
8+1			5	1								
7+3		1	4	1								
7+2	1	1	10	5	1							1
7+1	1	3	4	5	1						2	2
6+3	6	2	7	5	1							
6+2	14	14	18	15	9						2	19
6+1	23	18	4	4	4					4	14	20
5+3	12	9	14	4	8	2					1	9
5+2	22	23	4	6	23	2				6	16	21
5+1	14	23	2		10	2			8	14	15	6
4+3	14	7	3	4	20	2			5	8	3	3
4+2	9	14	6	12	19	14			21	17	17	25
4+1	5	10	6	5	12	2		1	12	26	13	13
3+3		1			8	4			11	5		1
3+2	1	3	1		1	24		8	5	7	18	5
3+1		3						5	11	2	6	1
2+3	1			2	3	2		5	7	4	5	1
2+2	2		3	2	7			24	27	1	5	2
2+1	5	1	1	1		1		4	6	1	1	1
1+3								14				
1+2								1	8			
1+1						1						

Conidiophores of the *Erysiphe polygoni* type characteristically form no spore chains and have a diurnal cycle of sporulation that may be represented by a more flattened curve (Tables 3 and 4). Only one spore, on the average, was abstracted during a 24-hour period as compared to 4 to 6 or more in the conidial-chain-forming powdery mildews observed. The cycle of sporulation found for *E. polygoni* on beans corresponded closely to that found for the same conidiophore type on *Euonymous*, and with that observed by Yarwood<sup>11</sup> for clover mildew, though Yarwood's data were presented in a different form. Abstraction of conidia (Tables 3 and 4) started about 10 a.m. and was completed by 2 p.m., or soon thereafter. Active discharge of conidia, as described by Hammarlund,<sup>10</sup> was not observed in

<sup>10</sup> See footnote 5.

<sup>11</sup> See footnote 6.

TABLE 2.—*Diurnal cycle of maturation and abstraction of conidia of the powdery mildew of rose, Sphaerotheca pannosa.* Each number represents the percentage of the binumerical type indicated, observed at each period of collection and observation. The highest number for each observation period is printed in bold-face type. The diurnal cycle is illustrated by the vertical fluctuation in bold-face numbers from 2 a.m. to 12 p.m.

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstraction of conidia											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
8+3												
8+2												
8+1		1.5	11.3									
7+3		2.9	5.6	8.3								
7+2	1.3	4.4	11.3									
7+1	2.6	13.2	11.3		2.3							3.6
6+3	2.6	7.4	11.3	22.2	4.6						4.2	
6+2	11.8	19.1	14.1	2.8	15.9						8.3	7.3
6+1	15.8	2.9	2.8	13.9	13.7					3.1	2.1	18.2
5+3	7.9	10.3	14.1	8.3	25.0						29.2	5.5
5+2	7.9	1.5	8.5	11.1	20.5					2.0	18.8	30.9
5+1	9.2	2.9		2.8	2.3	2.0			1.9	14.3	4.2	7.3
4+3	18.4	7.4	4.2	5.6	6.8				1.9	17.4	16.7	7.3
4+2	7.9	5.9	4.2	8.3	4.6	2.0			11.4	18.4	4.2	12.7
4+1	9.2	4.4			2.3	17.7			10.5	14.3	2.1	5.5
3+3	2.6	1.5			2.3	5.9			17.1	21.4	4.2	1.8
3+2	2.6	4.4				37.2	1.2	1.2	18.1	4.1	2.1	
3+1		5.9		2.6			13.8	8.4	8.6	7.6	1.0	
2+3		1.5		2.8			13.8	1.2	1.2	19.1	3.1	
2+2		2.9		2.6		3.9	18.1	4.8	7.6		2.1	
2+1				2.8			20.5	30.9	1.9	1.0	2.1	
1+3						3.9	19.3	11.1	1.9			
1+2							15.7	23.5				
1+1							15.7	18.3				

these studies, and abstracted conidia were commonly observed adhering to the conidiophore.

TABLE 3.—*Diurnal cycle of asexual spore maturation and abstraction in bran powdery mildew, Erysiphe polygoni, a non-chain former.* The numbers presented represent the actual numbers of each binumerical conidiophore type observed at each period of collection. The type most frequently observed is indicated by bold-face numbers

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstraction of asexual spores											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
1+5												
1+4												
1+3	4			1	5	5		1		1		
1+2	40	35	35	44	55	3		5	1	38	38	30
1+1	4			2	1			23	42	11	3	13
0+3	2						2	1				
0+2	4		4				32	43	1	1	14	1
0+1		4	4					4				

TABLE 4.—Diurnal cycle of maturation and abstraction of conidia in powdery mildew of *Euonymous japonicus*, a non-chain former. Data are presented as in table 3

No. of cells in conidio- phore exclu- sive of basal cell	Diurnal cycle of maturation and abstraction of conidia										
	← a.m. →						← p.m. →				
	2	4	6	8	10	12	2	4	6	8	10
1+5				1	1						
1+4	1		1	4					1		
1+3			1	1					2	3	
1+2	42	54	71	60	100	42	11	10	18	39	45
1+1	3	22	1	3			1	27	36	29	14
0+3			2				1	1			
0+2	5	3	1		1	43	34	10			
0+1	1	4					1				4

A graphical summary of the diurnal cycles of sporulation (Fig. 2) of the powdery mildews observed reveals that the chain-forming powdery mildews *Erysiphe cichoracearum*, *Sphaerotheca pannosa*, and *Podosphaera leucotricha* are similar in that their high phase of conidiophore development came about 8 a.m. and their low phase came about 2 p.m. The curve of conidiophore development of the non-chain-forming *E. polygoni* and *Oidium euonymi-japonici* (Fig. 2, Tables 3 and 4) differs in height and in contour from the curves of the conidial chain-forming mildews, while the latter differ among themselves mainly as to height of the high phase, as measured binumerically.

#### Diurnal Cycle of Spore Abstraction as Shown by Dislodgement of Conidia

In Fig. 3 are recorded graphically the results of spore-catching experiments with *Erysiphe cichoracearum*. Between the hours of 10 a.m. and 1 p.m. there was abundant abstraction of conidia, as is shown by the differences between the count at 10 a.m. and that at 1 p.m. and by the rise in the line between those two points. The diurnal cycle of maturation and abstraction of conidia, which is indicated by these spore counts for the powdery mildew of sunflower, is substantially similar to the diurnal cycle obtained by microscopic examination.

#### NUCLEAR DIVISION IN THE CONIDIOPHORE

Examination of the stained conidiophores of *E. cichoracearum* from sunflower revealed the frequent occurrence of two nuclei in the basal cell, and of two nuclei in the cell above it. The results of counts of the frequency of occurrence of conidiophores with two nuclei in the basal cell and of conidiophores with two nuclei in the cell above it (Table 5) indicate that nuclear division takes place in both cells but is the more active in the basal cell. These data suggest that both the basal cell and the cell above it may function as generative cells, as is indicated by Foëx's<sup>12</sup> drawings. As many

<sup>12</sup>See footnote 8.

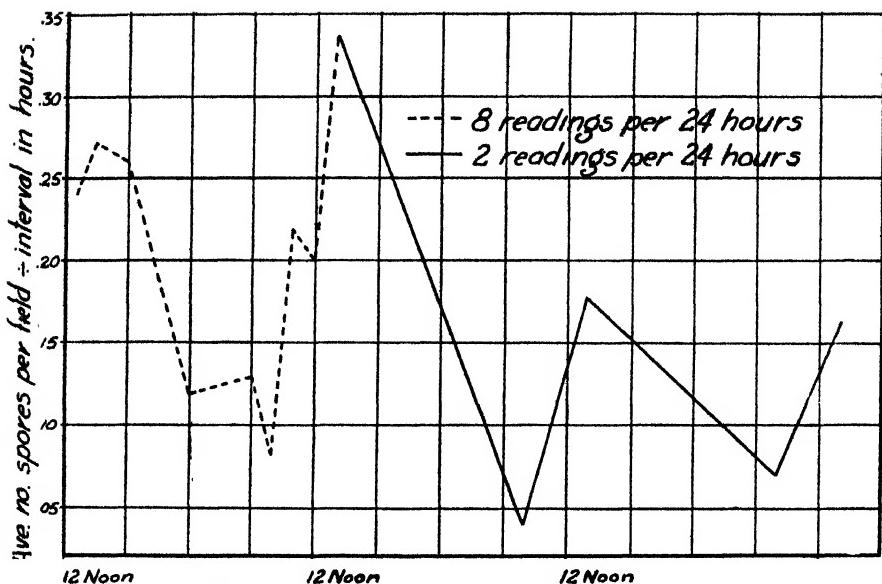


FIG. 3. Diurnal cycle of maturation of conidia of sunflower powdery mildew, *Erysiphe chichoracearum*. Each point on the graph indicates the number of conidia per low-power field per hour caught from the same excised leaves on 5 per cent sucrose. The leaves were snapped over a pint can at the bottom of which a glass slide had been placed. Each figure is based on from 125 to 175 microscope fields of  $2.14 \text{ mm}^2$ . From left to right an upward slope indicates abstraction of conidia and a downward slope indicates cessation or abstraction.

as 4 nuclei in a single cell of a conidiophore have been observed, but this is considered unusual.

No pronounced diurnal cycle is apparent from the data of table 5, but it is noteworthy that, at the time of the experiment (December 3rd), the number of divided nuclei and recently divided cells observed for the daylight period (9 a.m. to 6 p.m.) was more than double the number observed for the night period.

TABLE 5.—Nuclear and cell division of *E. chichoracearum* on greenhouse sunflowers, Dec. 3, 1938. The tabulated figures represent the number of conidiophores, out of approximately 200 observed at each period, bearing the type of cell designated

Time of collection of conidiophores	Conidiophores with 2 nuclei in		Conidiophores showing recent divisions in	
	Basal cell	Second cell	Basal cell	Second cell
9 a.m. .... .....	7	8	2	8
12 midnight .....	12	5	1	10
3 a.m. .... .....	13	3	3	6
6 a.m. .... .....	6	3	0	7
Total (for dark period) ..	38	19	6	31
9 a.m. .... .....	30	19	11	18
12 midday .....	22	8	27	4
3 p.m. .... .....	12	7	9	5
6 p.m. .... .....	12	15	2	6
Total (for light period) ..	76	49	49	38

## SUMMARY

Periodic microscopic examination of non-chain-forming powdery mildews of bean and *Euonymous* (*E. polygoni*-type conidiophores) revealed a diurnal cycle of conidiophore development similar to that reported for *E. polygoni* on clover. The period of abstraction of conidia occurred between 10 a.m. and 2 p.m. in both cases.

Periodic microscopic examination of the conidial-chain-bearing powdery mildews (*E. cichoracearum*-type conidiophore) of sunflower, rose, apple, aster, and cucumber revealed a more complex diurnal cycle of conidiophore development. Abstraction occurred between 6-8 a.m. and 2-4 p.m. and formation of the succeeding crop of conidia occurred between 2-4 p.m. and 6-8 a.m. in all cases.

In powdery mildew of the sunflower maximum spore abstraction occurred between 8 a.m. and 2 p.m. as shown by catching dislodged spores periodically over several days. A diurnal cycle of spore maturation and liberation, similar to that apparent from microscopic examination, was revealed.

Microscopic examination of stained conidiophores of sunflower powdery mildew revealed conidiophores with 2 nuclei in the basal cell and conidiophores with 2 nuclei in the cell next above the basal cell. This is believed to indicate that both cells may function generatively.

EXPERIMENTAL PRODUCTION OF BLACKFIRE ON TOBACCO<sup>1</sup>

E. M. JOHNSON, STEPHEN DIACHUN, AND W. D. VALLEAU

(Accepted for publication Aug. 7, 1939)

It is common knowledge that natural or artificial inoculation of tobacco leaves with *Bacterium angulatum* produces only a small, relatively harmless, angular leaf spot, and inoculation with *Bacterium tabacum* usually produces only the so-called "typical" halo wildfire spot on well-nourished, rapidly growing tobacco plants. In field epidemics of late-season blackfire<sup>2</sup> caused by either of these organisms, the spots are large, zonate, and destructive, particularly on topped dark tobacco. For a long time it has been recognized that there is a close correlation between the occurrence of blackfire epidemics and rainy, stormy weather, but it also has been observed frequently that tobacco in low areas may be destroyed by blackfire whereas tobacco growing on somewhat higher land may escape injury nearly completely. Frequently this condition occurs without storm injury. Clayton<sup>3,4</sup> has attempted to

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>2</sup> Blackfire is used in this paper to signify the concentric type of spot that occurs in wet seasons on topped dark tobacco. It may be caused by either *Bact. tabacum* or *Bact. angulatum*. The terms wildfire and angular leaf spot are reserved for the spots produced on tender tobacco leaves by the respective organisms. The etiological factors, aside from the parasite, which produce wildfire and angular leaf spot are so different from those causing blackfire that the latter may be considered a distinct disease.

<sup>3</sup> Clayton, E. E. Water soaking of leaves in relation to development of the wildfire disease of tobacco. Jour. Agr. Res. [U. S.] 52: 239-269. 1936.

<sup>4</sup> \_\_\_\_\_ Water soaking of leaves in relation to development of the blackfire disease of tobacco. Jour. Agr. Res. [U. S.] 55: 883-889. 1937.

show that water-soaking is a necessary factor for the development of what he has termed "epidemic" wildfire, and late-season blackfire. Valneau *et al.*<sup>5</sup> have questioned this point, and have shown that water-soaking cannot explain the outbreaks of blackfire as they occur on maturing dark tobacco in Kentucky.

It is the purpose of this paper to report the experimental production of the typical, zonate, late-season type of spot in the field, and in the greenhouse under controlled conditions.

#### FIELD STUDIES

In 1935, large, zonate spots, typical of those occurring late in the season on both white Burley and dark fire-cured tobacco, were induced on white Burley tobacco in an infertile area on the Station farm at Lexington with single-colony and mixed cultures of *Bacterium angulatum*. The late-season disease was induced also on topped and suckered dark fire-cured tobacco in western Kentucky with cultures of *Bact. tabacum* and *Bact. angulatum*.

Prior to 1935 numerous field tests with *Bacterium angulatum* and *Bact. tabacum* were made on tobacco plants growing in fertile soil but produced only the typical angular leaf spots and halo wildfire spots. The 1935 inoculations with *Bact. angulatum* indicated that the fertility of the soil may have a marked effect upon the susceptibility of tobacco to injury by these organisms because inoculations to Burley plants in fertile areas of the same field developed the usual small, angular, relatively harmless spots.

In 1938, inoculations were made with *Bacterium tabacum* and *Bact. angulatum* to dark fire-cured tobacco throughout the season in an infertile area in western Kentucky. Late in the season, after topping, similar inoculations were made in plots of high and medium fertility at Lexington. In the western Kentucky tests 37 plants of the 60 inoculated with *Bact. tabacum* developed the large, zonate spots, typical of those occurring in nature on topped dark tobacco (Fig. 1). On the same plot 3 plants of 60 inoculated with *Bact. angulatum* developed the late-season zonate disease. At Lexington, in the plot of medium fertility, 8 of 9 plants inoculated with *Bact. tabacum* and 2 of 9 plants inoculated with *Bact. angulatum* developed  $\frac{1}{2}$  to  $3\frac{1}{2}$  inch zonate spots. In the fertile plot at Lexington 3 of 9 inoculated with *Bact. tabacum* and 2 of 10 inoculated with *Bact. angulatum* developed the destructive zonate spot.

During the tests in western Kentucky rains fell on June 10, 11, 18, 19; July 2, 12, 17, 18, 19, 29, 30, 31; August 2, 3, and 11. The rains in June were not heavy and were unaccompanied by wind. Those of July 2 and 12 were heavy and were accompanied by high north winds. Despite the heavy and frequent rains of July and August water-soaking of leaves was never observed even though the winds broke many leaves. Leaves on the windward side of plants were often turned over and had on their under surfaces  $\frac{1}{2}$  to  $\frac{1}{2}$  inch bruised areas made up of numerous pinpoint water-soaked appear-

<sup>5</sup> Valneau, W. D., S. Diachun, and E. M. Johnson. Injury to tobacco leaves by water soaking. *Phytopath.* 19: 884-890. 1939.

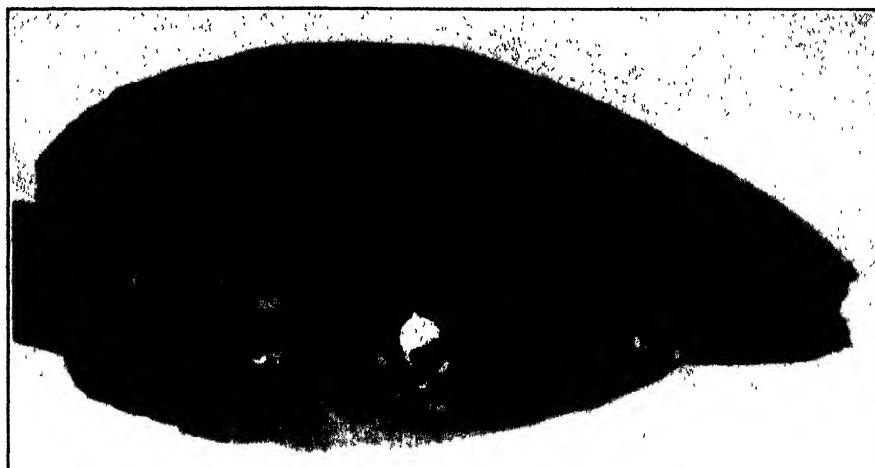


FIG. 1. Fourth leaf from the bottom of a topped plant of dark fire-cured tobacco, atomized in the field with *Bacterium tabacum*, June 15, 1938. (Dew was heavy every morning. Rain fell, June 16 and 18.) Photographed June 30, 1938.

ing spots (stippling), which usually disappeared in 3 to 8 hours. This stippling is not to be confused with incipient infections sometimes found around older spots. Many of these leaves were tagged and some were inoculated with *Bacterium tabacum* and *Bact. angulatum*. Comparable leaves without stippling were tagged and some were inoculated. The stippled leaves developed no more natural infection, nor did they develop more infection when inoculated than the nonstippled leaves.

During most of the tests, both in western Kentucky and at Lexington, dews were heavy and the plants remained wet until 9 or 10 a. m. On inoculated plants the dead centers of spots were, in early morning, surrounded by a narrow border of dark green to brown, wet, necrotic tissue sharply delimited from the inner dead, brown tissue and the outer, healthy area. During the day this wet, necrotic border dried out; if there was bright sunlight, it had a scalded appearance. In this way, spots gradually increased in size from day to day and coalesced, the final size often being limited only by contact with the midrib or larger veins. Heavy dews seem to be important not only in the increase in the size of spots but also in the development of new infections. On plants inoculated in the field, in the absence of rain, new infections have been observed on leaves directly under inoculated areas, during periods of heavy dews. It has been observed repeatedly that low areas in fields, in seasons of little rainfall, may develop blackfire, whereas the high areas may be entirely free. Plants in these low areas have been observed to remain wet much longer in the morning than those on the higher areas. Fogs may be important. It is not unusual to observe fog pockets in the early evening in many fields.

#### GREENHOUSE STUDIES

In conjunction with the field studies, experiments were carried on in the greenhouse with low-topped Burley and dark tobacco plants inoculated with

pure cultures of virulent single-colony isolates of *Bacterium tabacum* and *Bact. angulatum*. Two preliminary experiments showed that wildfire infection on untopped and topped plants kept dry was of the typical halo kind, while spots on topped plants kept in an artificial fog produced by an atomizing spray nozzle operated by steam were larger, were surrounded by a dark green-gray, wet border, and usually were without halos. These spots increased in size and often coalesced to form large, dead areas made up of several concentric spots. If the inoculated topped plants were kept in the fog continually the spots were not zonate, but if kept in the fog during the night and allowed to dry in the daytime zonate bands developed.

On November 29, 1938, three low-topped Burley plants and three untopped plants of the same age, in 8-inch pots, were inoculated with 24-hour broth cultures by atomizing; the atomizer was held  $\frac{1}{4}$  of an inch from the lower leaf surface. The left side of each leaf was inoculated with *Bacterium angulatum* and the right side with *Bact. tabacum*. Two topped and two nontopped plants were placed nightly from 5 p. m. until 8 a. m. in the fog, which kept the leaf surfaces moist. Water-soaking was never observed on these plants. A topped and a nontopped plant were placed at one end of the greenhouse away from the fog. By December 5 infections caused by both *Bact. tabacum* and *Bact. angulatum* were destructive on the 3 upper leaves of the 2 plants kept in the fog nightly. Spots were coalescing to form dead areas  $\frac{1}{2}$  to 1 inch in diameter. There was but little difference between the spots caused by *Bact. tabacum* and those caused by *Bact. angulatum*. On the nontopped plants in the fog and the plants kept dry both wildfire and angular leaf spots were of the typical relatively harmless type.

By December 6 some of the spots on the topped plants kept in fog nightly showed two or three distinct zones, the outermost one being a necrotic, wet border.

Every night until December 10, when the experiment ended, another band or zone was added to the spots on the topped plants kept in the fog at night. In the morning the new zone was wet, necrotic, grey-green or light brown, and not sunken. During the day, when the spray was turned off and the leaf surface became dry, the zone became dry, sunken, and brown, usually with a sharp dark brown line of demarkation between the dead tissue and the healthy green tissue, with but little if any increase in size of the spots during the day.

The necrotic spots on the topped plant not in the fog were not so large as those on the topped plants kept in the fog; they were not zonate. The spots on all the nontopped plants were of the familiar typical halo wildfire, and small angular-leaf-spot type.

This experiment was repeated on January 4, with similar results. In figure 2 is shown the actual daily increase in size of spots produced when leaves inoculated with *Bacterium angulatum* and *Bact. tabacum* were placed in the fog during the night and allowed to dry off during the day.

Twice, low-topped dark-fired plants were atomized with *Bacterium tabacum*. Both times, large, zonate spots, such as those shown in figure 3 de-

veloped on the plants kept in the fog. In one of these experiments one side of each leaf was atomized with *Bact. angulatum*. The resulting infections developed much more slowly than the spots on the same leaves caused by *Bact. tabacum*, and only a few of the spots on the side of the leaves inoculated with *Bact. angulatum* became large and zonate.

Two experiments were performed in which inoculation with *Bacterium tabacum* and *Bact. angulatum* was made by needle prick rather than atomizing. In neither case were such inoculations very successful in producing blackfire but a small per cent of the infections did produce rather large, zonate spots.

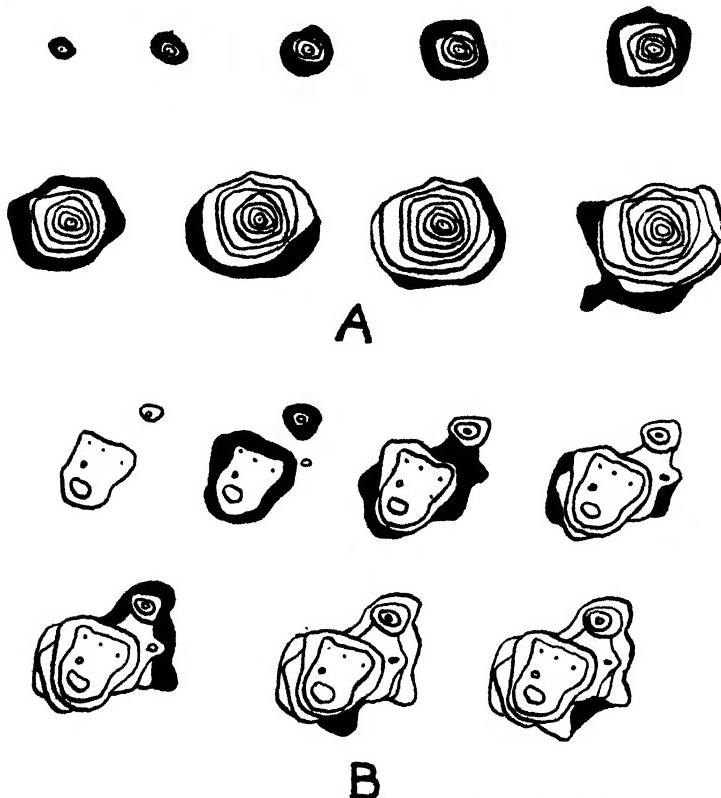


FIG. 2. A. Daily increase in a typical zonate blackfire spot produced by *Bact. angulatum* when the inoculated plant was placed in a fog nightly. The black outer zone was necrotic and wet in the morning, when the drawing was made. B. Daily increase in size of a zonate blackfire spot produced by *Bact. tabacum* under the same conditions.

#### DISCUSSION

Although it has been believed for some time that rainy weather is in some way connected with the rapid development of blackfire outbreaks in the field, the zonate blackfire type spot has not previously been produced experimentally by inoculation with pure cultures of either *Bacterium angulatum* or *Bact. tabacum*. Consequently it was not definitely known that either of these organisms was necessarily concerned in outbreaks of the disease on topped dark tobacco, and the relation of wet weather to outbreaks was not



FIG. 3. A. Blackfire on a topped dark tobacco plant atomized with *Bact. tabacum* 6 weeks after topping. Photographed 11 days after inoculation. B. Close-up view of several zonate spots on the same plant showing coalescence of spots. Photographed 22 days after inoculation.

clearly understood. The theory has been advanced<sup>6</sup> that water soaking of leaf tissues during storms is necessary to break down the resistance of tobacco leaves to *Bact. tabacum* and *Bact. angulatum* in the natural development of the disease in the field. But our observations during outbreaks of blackfire on dark tobacco have indicated that, whereas there might sometimes be a marked increase in injury following a severe storm, yet the zonate blackfire spots frequently developed in the absence of storm injury and in the absence of conditions likely to bring about water soaking of the leaves. Furthermore, isolation studies from the advancing edges of blackfire spots showed these tissues to be largely sterile. The experiments here reported show that large zonate spots can be produced on low-topped Burley and dark tobacco plants in the field, and in the greenhouse, in the absence of water-soaking. In these experiments it was found that if leaves atomized with a virulent isolate of *Bact. tabacum* were kept moist during a part of the day by placing the plants in an artificial fog, large, zonate spots typical of those occurring in natural epidemics were produced.

Thus far, only relatively virulent isolates of the organisms have been used. Whether weak strains would produce the same results is not yet known. In these studies *Bacterium tabacum* usually caused larger and more destructive spots than *Bact. angulatum*. Sometimes *Bact. tabacum* produced large spots when *Bact. angulatum* failed to do so on another part of the same leaf. It may be that the isolate of *Bact. angulatum* used was not sufficiently virulent, and that more virulent strains might be more nearly comparable to the virulent isolate of *Bact. tabacum* in the production of large zonate spots.

#### SUMMARY

Large, zonate spots very similar to those occurring in natural late-season epidemics of blackfire were produced on Burley and dark tobacco plants in the field by atomizing leaves with *Bacterium tabacum* and *Bact. angulatum*. Dew usually covered the inoculated leaves every morning.

Similar large, zonate, destructive spots were produced on topped Burley and dark tobacco plants inoculated with virulent isolates of *Bacterium tabacum* and *Bact. angulatum* when the plants were kept nightly in an artificial fog which formed a film of water on the leaves.

Both in the field and in the greenhouse the spots increased in size during the night, when a wet, necrotic, advancing border was formed that became dry and brown during the day.

Large dead areas 3 to 4 inches in diameter were formed by increase in size of individual spots and coalescence of adjacent spots.

This appears to be the first report of the experimental production of the zonate blackfire spots by pure culture inoculations and under controlled conditions.

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<sup>6</sup> E. E. Clayton. *Loc. cit.*

# TIME OF GROWTH<sup>1</sup> OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA AT RHODODENDRON, OREGON

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(Accepted for publication July 14, 1939)

## INTRODUCTION

The seasonal fluctuations in growth rate of white pine blister rust (*Cronartium ribicola* Fischer) cankers on western white pine (*Pinus monticola* Douglas) were studied at Rhododendron, Oregon, from 1934 to 1937, inclusive. Lachmund<sup>2</sup> and Buchanan<sup>3</sup> determined the annual growth rate of blister rust cankers in British Columbia and Idaho, and Lachmund<sup>2</sup> found that canker growth is less in winter than during the growing season. Rhoads<sup>4</sup> observed that growth of the cankers on *Pinus strobus* L. during the latter part of the summer was twice as fast as that during the spring and early part of the summer of 1918 at Kittery Point, Maine. The study herein reported was made to secure information on the time of year and at what relative rates growth of blister rust cankers takes place, and thereby to gain a better conception of the phenology of this important parasite.

## THE STUDY AREA

The area selected for the study is on the west slope of the Cascade Mountains, about 10 miles southwest of Mount Hood, at Rhododendron, Oregon. This location is approximately at the latitudinal center of the botanical range of *Pinus monticola*, and at this latitude the species ranges from near sea level to about 5,000 feet elevation. The study was made in a typical stand of reproduction from 10 to 20 feet in height, growing on nearly level ground at an elevation of approximately 1,650 feet and well within the natural range of western white pine in that region. The trees used were typical in thrift for the locality and of average size for the stand.

## METHODS

Cankers were chosen for study by selecting as nearly as possible a relative representation of all cankers occurring on the trees used. That is, an equal percentage of existing branch cankers and stem cankers were chosen, and also a proportional number of existing primary- and secondary-branch cankers. The numerical basis employed in each diameter class was approximately proportional to the natural frequency of cankers as found on the

<sup>1</sup> The term "growth" of cankers is used to designate the extension of the discoloration of the bark, which accompanies and bears a fairly constant relation to the growth of the mycelia in the bark.

<sup>2</sup> Lachmund, H. G. Growth and injurious effects of *Cronartium ribicola* cankers on *Pinus monticola*. *Jour. Agr. Res. [U.S.]* 48: 475-503. 1934.

<sup>3</sup> Buchanan, T. S. Annual growth rate of *Cronartium ribicola* cankers on branches of *Pinus monticola* in northern Idaho. *Phytopath.* 28: 634-641. 1938.

<sup>4</sup> Rhoads, Arthur S. Studies on the rate of growth and behavior of the blister rust on white pine in 1918. *Phytopath.* 10: 513-527. 1920.

area. An effort was made to secure cankers that were of average health and vigor, that would remain alive for at least one year, and that would not coalesce with another canker before the experiment was completed. Cankers in all stages of development, from the first incipient discoloration to those that had produced aecia several times, were used. Cankers were selected in all parts of the crowns in an effort to obtain a true sample of those subjected to all existing local influences.

At the start of the experiment the limits of discoloration on each canker were marked with paint, as described by Buchanan,<sup>5</sup> and the following data were recorded for each canker: Canker number; type of branch or stem; location in the tree; the year's growth upon which the canker originated; the total length of the canker; the diameter of the branch or stem at both extremities of the canker; the stage of development; and the general condition of the canker. These data were used both in judging the general representation of the samples and in attempting correlations of probable influences with differences in time of growth for individual cankers.

Growth measurements were secured on two series of cankers. In Series 1, 52 cankers, on 12 trees, were marked on March 8, 1934, and were measured periodically until June 5, 1935. In Series 2, 65 cankers, on 7 trees, were marked on March 6, 1936, and measured periodically until May 12, 1937.

In preparation of the study plan it was believed that growth measurements taken at monthly intervals<sup>6</sup> throughout the year would be sufficient to show the time of growth. Soon after the experiment was under way, however, it became evident that at certain times in the year growth measurements at shorter intervals would be necessary. Accordingly, measurements were taken at 2-week intervals on the cankers of Series 1 from early November, 1934, to early June, 1935. The cankers in Series 2 were measured at weekly intervals from the time of marking to early May; and at monthly intervals thereafter, except during the periods of growth retardation in the fall of 1936 and of the beginning of growth acceleration in the spring of 1937, when measurements were taken at weekly intervals.

Just as in similar studies by Lachmund and Buchanan<sup>7</sup> the extremities of the cankers were considered to be at the limits of the discoloration of the infected bark. At the time of each measurement the total growth, since the time of marking, toward the distal end of the branch or stem was considered as growth upward, and the total growth, since the time of marking, toward the proximal end as growth downward. Growth measurements were carefully taken with vernier calipers registering to the nearest hundredth of an inch.

#### RESULTS

There were great differences in time of growth between individual cankers. All cankers ceased perceptible growth for a period of at least 1

<sup>5</sup> See reference in footnote 3.

<sup>6</sup> The intervals between measurements were not always exactly one month, two weeks, or one week, but were approximately so.

<sup>7</sup> See references in footnotes 2 and 3.

month during the year. Some stopped growing as early as September, and others continued growth until December. Most cankers showed no growth during January. However, there was no period of a month's duration during which all cankers showed no growth. Appreciable growth usually commenced in late March, but many cankers showed no growth until April and some did not grow until May. The individual canker that grew continu-

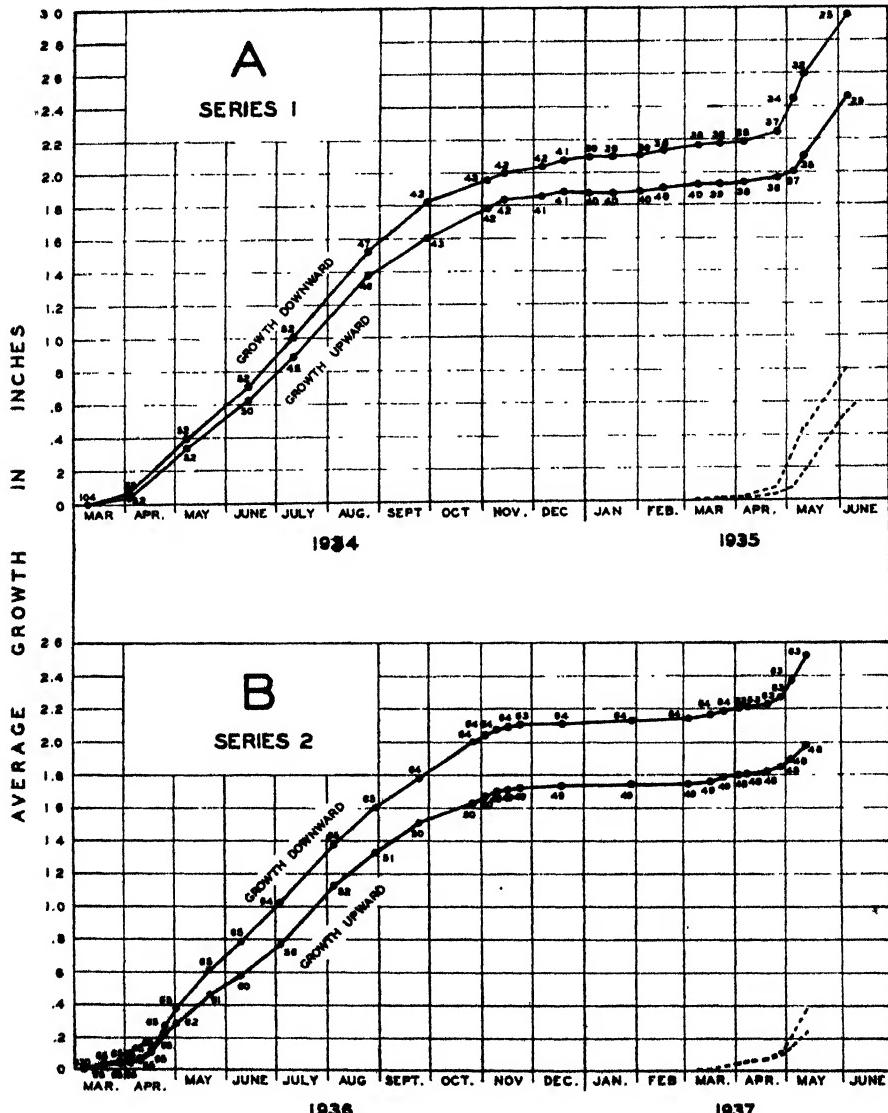


FIG. 1. The average cumulative growth, both upward and downward: A, for cankers in Series 1 during the course of observations from March 8, 1934, to June 5, 1935; and B, for cankers in Series 2 from March 6, 1936, to May 12, 1937. The dates on which measurements were taken are indicated by the positions of the points on the graph, and the small numeral at each point gives the number of cankers on which it was based. For convenience in comparing spring growth, the terminal portions of the graphs are repeated, by dotted lines, starting at zero on March 8, 1935, and March 8, 1937.

ously for the greatest number of months showed detectable growth for a period of 11 months, while at the other extreme 1 canker showed no growth for a period of 6 months. To a lesser extent differences were found between the time of growth of the 2 ends of individual cankers. For example, cankers were noted on which upward growth stopped in the early fall, while growth downward continued slowly for a month or more.

The data were analyzed in an effort to correlate peculiarities of time of growth of individual cankers with the various factors that were believed to have some influence on time of growth. No apparent correlation was found between time of growth of individual cankers and any one of the following factors: Age of canker; stage of canker development; general health of canker; type of canker (stem cankers, primary-branch cankers or secondary-branch cankers); size of cankered branch or stem; location of canker in the tree; or size of tree.

The seasonal fluctuations of canker growth are illustrated in figure 1, which shows graphically the growth, both upward and downward, for cankers in both series during the course of the observations.

In figure 1 it may be noted that after the start of a series certain cankers were eliminated from the basis. The principal cause of cankers' becoming

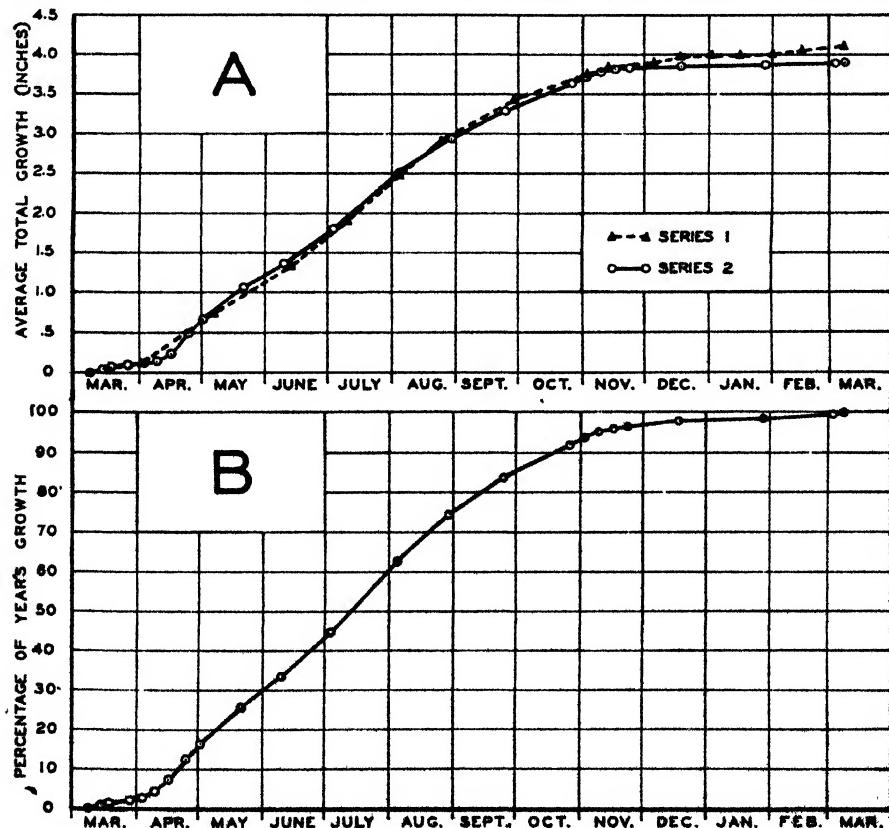


FIG. 2. Growth of cankers from March 8 to March 8: A, average cumulative total growth in each series; B, average cumulative percentage of total growth in both series.

unusable, especially for measurements of upward growth, was "flagging" (death of the branch beyond the canker). Two other important causes were coalescence with other cankers and growth of cankers into nodes or whorls where the discoloration limits could not be seen. Certain small irregularities in the graphs may be accounted for by these changes in the bases.

The combination of upward growth and downward growth is described as the total growth of a canker. The average cumulative total growth of cankers for Series 1 is compared with that of Series 2 in figure 2, A, showing the similarity of growth fluctuations and amount of yearly growth for the two series. In figure 2, B, the two series are combined to show the average cumulative percentage of the total growth for a 1-year period.

#### DISCUSSION

The period over which canker growth occurred is shown in figures 1 and 2. All of the graphs show that the growth was greatest from April to November and that there was little growth during the winter months. From figure 2, B, it may be seen that 90 per cent of the year's growth took place from April 1 to November 1, and that only about 2.5 per cent of the year's growth occurred in December, January, and February. Most of the cankers showed no growth during the winter months. Although the average curve shows a slight growth throughout the winter, each of the cankers stopped perceptible growth for at least a month during the winter and most of them for 2 or 3 months or longer. The results agree with Lachmund's<sup>8</sup> findings that canker growth on *Pinus monticola* is less in winter than during the growing season. Rhoads'<sup>9</sup> observations that canker growth on *P. strobus* during the latter part of the summer was twice as fast as that during the spring and early part of the summer are in contrast with the findings for *P. monticola* reported by Lachmund, as well as the results herein reported.

One factor that showed some apparent correlation with the time of canker growth was temperature. Although no daily temperature records are available for the immediate vicinity of the study area, the general monthly records for the region indicate that temperature was one of the factors governing the period over which canker growth occurred. The weather records show that the spring of 1934 was unusually warm. Following the mildest December of record, January, February, March, and April, all established new records for high mean temperature. The effect of this unusually warm spring is manifested in the growth curves of figure 1 when comparison is made with the more nearly normal weather of the 3 following springs. The springs of 1935 and 1937 were somewhat below normal in temperature. January of both these years was unusually cold—January, 1937, being the coldest month of record in Oregon. These cold periods in January caused a severe check of vegetative growth in both years. In 1935 the beginning of spring was rather cold. March was only 1° warmer than February, and both March and April had temperature below normal, that of April being the lowest mean for that month since 1929. April of 1937

<sup>8</sup> See reference in footnote 2.

<sup>9</sup> See reference in footnote 4.

was cool and unusually cloudy, having the greatest number of cloudy days of any April since 1915. The effect of this cool weather in April of both 1935 and 1937 may be seen on the graphs in figure 1 when compared with the nearly normal spring of 1936 and the unusually warm spring of 1934.

The falls of 1934 and 1936 were both somewhat warmer than normal, although a sudden cold period, commencing the first of November in 1936 and lasting most of that month, shows some effect on the period of rapid canker growth as depicted by the graphs in figure 1. (Compare the sudden leveling off in November of the growth curve for 1936 with the gradual leveling off of the growth curve for the fall of 1934.)

In each of the 4 springs during the course of this study, the breaking of peridia on the aecia-producing cankers throughout the general vicinity synchronized very well with the beginning of appreciable growth of cankers of all stages. In each case perceptible growth started at about the time the first peridia were broken, and by the time most peridia were broken rapid canker growth was under way. Another consistent indicator of time of canker-growth acceleration was the breaking of buds on deciduous trees in the vicinity of the study area. Rapid canker growth ceased in the falls of both 1934 and 1936 at about the time the deciduous trees in the vicinity dropped their leaves. It is believed that these indicators of the beginning and the end of rapid canker growth may be applicable in estimating the period of rapid canker growth in other localities.

#### SUMMARY

A total of 117 white pine blister-rust cankers on 19 western white-pine trees were used in a study of the seasonal fluctuations of canker growth rate at Rhododendron, Oregon. Two series of cankers were used, one series in 1934 and 1935 and the other in 1936 and 1937. Canker growth curves are shown. Great differences in time of growth were found between cankers, and to a lesser extent between the two ends of individual cankers. Growth was rapid from April to November and ceased for 1 to 3 months or more during the winter. About 90 per cent of the annual growth took place from April 1 to November 1, and only 2.5 per cent occurred during December, January, and February. Temperature is believed to have been one of the factors governing the time of rapid canker growth. The time of breaking of the peridia on blister rust cankers and the time of the breaking of buds of deciduous trees in the spring are considered as probable indicators of the time of canker growth acceleration, while the time of leaf-fall of deciduous trees in the fall is suggested as a probable indicator of the time of canker-growth retardation.

BRANCH OFFICE OF THE

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MAINTAINED AT PORTLAND, OREGON, IN

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## PHYTOPATHOLOGICAL NOTES

*A Method for Testing Resistance of Tomatoes to Fusarium Wilt.*<sup>1</sup>—Many tests have been made of the varietal susceptibility of tomatoes to *Fusarium bulbigenum* var. *lycopersici* Wr. and R. for the purpose of selecting wilt-resistant varieties and strains adapted to particular conditions. These tests usually have been conducted under field conditions in soil known to be infested with the wilt organism. Edgerton<sup>2</sup> and, later, others planted directly in naturally or artificially infested soil, to eliminate susceptible individuals. In many cases the seedlings have been transplanted to infested soil in trays or cold frames. This technique permits the study of a larger number of plants than would be possible if they had been grown in clean soil and set in infested fields. Wager<sup>3</sup> and others have inoculated tomato plants by placing mycelium in holes at the base of the plants. This, however, is laborious and requires large amounts of inoculum if many seedlings are to be inoculated.

A method of inoculating thousands of young tomato plants was used with marked success in the spring of 1939 at the Tomato Disease Laboratory at Yoakum, Texas. The roots were immersed for periods of from 5 to 10 minutes in 4-7-day-old liquid nutrient cultures of *Fusarium bulbigenum* var. *lycopersici*. The cultures had been agitated daily to insure uniform distribution of the fungus through the inoculum. The plants were immediately transplanted to flats or cold frames. All tests were conducted out of doors from February through May. In the first experiment, seven-week-old plants from a large number of resistant and susceptible varieties were inoculated in this manner on February 24. The first definite symptoms were observed on March 15. Of the 1200 plants used in this test only 33 lived long enough to produce any seed and none of them were free of the vascular discoloration so characteristic of tomato wilt. Many of the resistant commercial varieties were included in this test. The reaction of the susceptible Gulf State Market and the resistant Louisiana Pink and Louisiana Dixie varieties is illustrated in figure 1. All of the plants in Gulf State Market variety have succumbed, while most of the plants in the other two still are apparently healthy. However, many of these plants later died from wilt.

Two other tests were conducted in which the roots of 8255 young plants also were immersed in a culture of the wilt fungus. There were 168 different samples of tomatoes, most of them being standard varieties. A few, however, were from a collection made by H. L. Blood, in South America, in 1937-38, and obtained through L. R. Hawthorn, horticulturist at the Winter Haven substation. The first distinct symptoms of tomato wilt appeared in 10 days in one test and in 16 days in the other. The relative resistance of the different varieties and strains was obtained by making counts at 10-14-

<sup>1</sup> Published with the approval of the Director as contribution No. 544, Technical Series, of the Texas Agricultural Experiment Station.

<sup>2</sup> Edgerton, C. W. A study of wilt resistance in the seed bed. *Phytopath.* 8: 5-14. 1918.

<sup>3</sup> Wager, V. A. *Fusarium* wilt in tomatoes in South Africa. *So. Afr. Jour. Sci.* 30: 240-246. 1933.



FIG. 1. Photograph taken 25 days after inoculating young tomato plants by immersing the roots for 10 minutes in a liquid nutrient culture of *Fusarium lycopersici*: Two strains of Gulf State Market in the tray on the left, Louisiana Dixie and Louisiana Pink in the tray on the right. Only 6 plants from this tray produced seed and they were of the Louisiana Pink variety.

day intervals. The plants were classified according to the severity of the disease. On the final count the stems of those plants not showing pronounced symptoms were cut and examined for internal discoloration. Only 5 plants from the standard varieties failed to show the vascular discoloration, while a number of plants in several of the South American lots were free of the disease.

The wilt-resistance ratings obtained from these counts were compared with the ratings obtained under severe wilt conditions in the field. In nearly all cases the ratings were in the same order, except that those for the plants artificially inoculated were generally somewhat lower, indicating that this method may be a more selective one than the setting of plants in infested fields.

The technique herein described for inoculating tomato plants is proposed as an aid in the selection of wilt-resistant tomatoes and in the study of strains of the fungus, since it is simple, accurate, and rapid. Readings on wilt resistance can be made within 4 weeks after inoculation if conditions are favorable for the development of tomato wilt. The method is similar to the one mentioned by Weindling and Armstrong<sup>4</sup> for inoculating cotton with the cotton wilt fungus.—A. L. HARRISON, Tomato Disease Laboratory, Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, A. and M. College of Texas, Yoakum, Texas.

<sup>4</sup> Weindling, R., and Armstrong, G. M. A water-culture infection method used in the study of *Fusarium* wilt of cotton. *Phytopath.* 29: 23. 1939.

*Unusual Bacterial Spot Symptoms on Peach Leaves.*—An aggravated case of the bacterial spot disease (*Bacterium pruni* E. F. S.), with unusual leaf symptoms, was observed near Springdale, Arkansas, on June 1, 1939. All stages of leaf-spot development were present in the 18-month-old Elberta orchard, but, instead of culminating in the typical shot-hole effect, large areas of the leaves became completely infiltrated with bacteria (Fig. 1, A). These affected areas, in some cases involving as much as one-half of the leaf, had a greenish-yellow translucent appearance when viewed by transmitted light, but were dark brown under reflected light. The ground beneath the trees was littered with leaves showing these atypical bacterial-spot symptoms, and other affected leaves (Fig. 1, B) remained in the trees, detached from the twigs, but adhering to adjacent leaves by the mixture of gum and bacteria, which oozed from the affected portions.



FIG. 1. A. Elberta peach leaves with different amounts of the leaf area invaded by *Bacterium pruni*. B. Diseased leaves attached to other leaves by the mixture of gum and bacterial exudate, which oozed from the infiltrated tissues.

Microscopic examination of the infiltrated, translucent areas revealed a complete disorganization of the cellular structure of the leaf. The small veins, which ordinarily delimit the size and shape of the spots, did not act as barriers to the spread of the organism. Pure cultures of the organism were secured from the infiltrated tissues.

These unusual symptoms were much more pronounced in two-thirds of the 20-acre block, where string beans had been grown between the peach rows in 1938 and the dried bean vines had been plowed under in March, 1939. In the balance of the block, where the beans had not been planted in 1938, the disease, while present, was running a normal course with only moderate defoliation and only an occasional leaf showing the infiltrated areas.—  
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Crops and Diseases, U.S.D.A., Washington, D. C., cooperating with the Arkansas Agricultural Experiment Station.

*A Blight of Wild Cherry Seedlings.*—A blighting of wild cherry (*Prunus serotina* Ehrh.) seedlings has been under observation since 1924. The disease, caused by *Sclerotinia scaveri* Rehm, appears each spring about the time the second pair of true leaves unfolds. The first symptom is the development of a brown, water-soaked region near the apex of the stem. This condition is accompanied by a loss of turgor and the infected seedlings (Fig. 1) are



FIG. 1. Blighted wild-cherry seedlings showing the characteristic drooping of apical portion of stem. Healthy seedling at the left.

readily detected by the characteristic drooping of the affected portion of the stem. The infection spreads from the stem into the leaves through the petiole and mid-rib. The basal portion of the leaf turns brown and finally the whole leaf is affected, assuming a bleached grey color. Conidial masses frequently develop on the leaves. The fungus continues to spread down the stem and, when it reaches the ground line, the young plant dies.

The disease was observed at Fort Valley, Georgia, from 1924 to 1928 and subsequently has been observed in the vicinity of Fayetteville, Arkansas.

TABLE 1.—Number of wild-cherry seedlings blighted in three quadrants on April 4, 1928

Quadrant number	Total number seedlings	Number healthy	Number blighted	Per cent blighted
1	147	55	92	62.5
2	88	58	30	34.0
3	250	149	101	40.0

In 1928, 3 quadrants, each 1 sq. m. in area, were laid out at random under a large tree near Fort Valley, Georgia. Although the total number of seedlings blighted during the period of seedling germination was not ascertained, the counts made on April 4 (Table 1) show from 34 to 62.5 per cent of the seedlings affected on that date. It is evident from these figures that the disease must be considered as a factor limiting the reproduction of *Prunus serotina* in the South.—JOHN C. DUNEGAN, Fayetteville, Ark., Division of Fruit and Vegetable Crops and Diseases, U.S.D.A., Washington, D. C., cooperating with the Arkansas Agricultural Experiment Station.

### BOOK REVIEWS

MELHUS, IRVING E., AND GEORGE C. KENT. *Elements of Plant Pathology*. 493 pages (including glossary, list of books and index), 259 figures. The Macmillan Company, New York. 1939. \$4.00.

This book, printed in larger type and on whiter paper than most texts of recent date in the field of applied botany, is timely in its appearance and of more than passing interest for the plant scientist and student. As stated by the authors in their Preface, "The chief contribution of the book lies in the emphasis placed on parasitism in disease processes and the principles relating to control measures, coupled with the condensation and omission of unnecessary morphological and mycological data." The text was not prepared to answer, nationwide, the needs of instructors in plant pathology but, on the other hand, it should be on the reference shelf of all research workers and teachers in botany and plant pathology and available to all students in these fields of science. As a text book for use in colleges and universities, it could be appropriately adopted over a large part of the United States with very little supplementation or adaptation.

It is approximately  $6 \times 8\frac{1}{2} \times 1\frac{1}{2}$  inches in size, substantially bound in plain green buckram, stamped on back and cover with the title in gold letters on a dark rectangular background. The contents consist of an introduction and 15 additional chapters dealing with plant pathology, 6 pages of glossary, a list, covering more than 4 pages, of books including author, title, publisher, year, and number of pages, dealing with some phase of plant pathology, and 17 pages of index in which bold-face type indicates illustrations. About 20 per cent of the text figures (51 of a total of 259) are diagrams of the host relation of the various parasites, indicating the active and dormant parts of the life cycles of the parasites and, in most instances, their parasitic and saprophytic rôle. These figures are of particular interest to the student in illustrating relationships where more than one spore form exists and where alternate hosts are included. The line drawings are well chosen and excellently reproduced, whereas the remaining illustrations, generally good, too frequently indicate lack of good material from which to choose and poor reproduction, resulting in difficulty of interpretation by the student.

The first 21 pages deal with introductory remarks and more or less of the historical background of this science, which is further developed in the next 46 pages under the headings of symptoms, parasitism and influences of environment on plant disease. The principles of control measures are set forth in an equal number of pages where rotation, sanitation, protection, quarantine, eradication, and resistance are described in varying degrees of detail. The first, second and third are considered under temporary and the remaining three methods under permanent control. The information on disease control is up-to-date with historical jottings and tabular data. The chemical reactions may be somewhat specialized and advanced but are not out of place.

Eight specific diseases caused by phycomycetes are described on the 45 pages in Chapter 8, including no bibliography or references. The diseases presented are caused by parasites covering the full range of variation of the fungi generally thought to be properly included in the group indicated and the authors are to be commended on their selections.

There are 10 diseases of plants caused by parasites described on 47 pages in Chapter 9, under the title of Diseases Caused by Bacteria. Mycological principles have been cut to the bone in relation to the organisms included in this chapter. The explanation of why a bacterial pathogen may have 2 or more scientific names is presented, but is difficult to understand and certainly unsatisfactory to a beginner. The diseases discussed are fairly well selected from a geographical viewpoint and also from the viewpoint of variable sources of inoculum and methods of dissemination.

The 10th chapter, covering 44 pages, deals with "virus-diseases" of plants. An introduction and historical review of the subject and its development, economic impor-

tance, symptoms, causal agent, properties of viruses, environmental, physical and chemical influences upon them, their movement, multiplication, vectors, dissemination, and control occupy the greater part of the chapter. The remainder deals with 4 well-selected plant diseases in which general statements made concerning "virus-diseases" are specifically applied.

The ascomycetes and 11 diseases caused by them on fruits, cereals, vegetables, field crops, and trees are dealt with in Chapter 11 and occupy 58 pages of concentrated, informative, and descriptive material. The selection of diseases and causal parasites is very representative with this group. The range of hosts affected is fair and proportional. Certain substitutions might be suggested, but the improvement would be only for limited geographical locations. The information presented is readily adaptable if necessary, but in most instances deals with the most economically important diseases, which, without saying, are also those upon which the most research has been done and about which most is known.

The imperfect fungi and diseases caused by 5 of them follow in the next 33 pages, constituting Chapter 12 of this book. The authors state that, "the imperfects induce more local and general necrosis . . . than any other group of symptoms," and, "most of the imperfects attack the aerial parts of plants"; yet, in their selection of 5 representative diseases, we find 3 of them caused by root infections of the host in the soil with similar secondary symptoms. The pathogenicity of a certain parasite was demonstrated "in 1908" and upon turning the page, lest the student forget, he is reminded again of the same fact. Likewise it is stated that a parasite forms "mats 2 to 12 inches in diameter," and a page or two later this information is repeated; again we find a "fungus carried on the seed," and less than 10 lines later, "the seed carries the organism." Why the losses caused by the cowpea root-knot organism should be added to the losses caused by the cotton wilt organism for quotation requires further explanation. These fungi, so frequently encountered, so variable in forms, so difficult for the student, and so inviting to the investigator apparently have not received the careful attention they in their own right deserve.

The basidiomycetes, divided under the 3 headings, Smuts, Rusts, and Wood and Root Rots are treated within the 85 pages of Chapter 13. The smuts are represented by 5 parasitic species in 3 genera, the rusts by 5 species in 3 genera and the wood and root rots by 3 parasitic organisms.

The treatment of the group, as a whole, is probably the best in the book and shows that first-hand acquaintance produces superior knowledge and more unified, complete and convincing description. The host-relation diagrams are particularly of interest and value in showing clearly to the student the complicated life cycle of these parasites. The illustrations are usually good. Names of spore forms are uniformly used, except where "basidiospore" appears in place of "sporidia." The general descriptive matter is definitely condensed and inclusive except, for instance, where the word "flower" appears 3 times in a 24-word sentence.

Diseases caused by seed plants and nematodes are presented in the next two chapters on 23 pages. The parasites and host range indicated are rather inclusive but severely condensed, probably because of the lack of their importance economically or because of the scarcity of these diseases in the central plains section of the country.

The final chapter deals with nonparasitic agents in relation to plant disease and it is indeed meager. The authors might have made a more variable selection for presentation. Four diseases are described, 3 of which are on apple, the fourth being a deficiency disease. A fair idea is obtained regarding the conditions that contribute toward their development and correction. Possibly this chapter could have been combined with Chapter 6, entitled "The influence of environment on plant disease."

The book is up-to-date and contains an accumulation of heretofore nonsummarized published data that gives it a definite, fact-containing, valuation seldom equalled. The use of words in this text is of special, atmosphere-giving interest and will be noticeable to all who read it. A few selected at random are here presented: ameliorated, dendritic, dirt, ephemeral, fuzzy, infectivity, mycelial, rattled, smutty, stoppage, tilth and viruliferous. The arrangement of the groups of diseases caused by classes of fungi, the bacteria, viruses, etc., is somewhat irregular when compared with previously published texts in plant pathology; but if this sequence has proved better in practice then it is fully justified.

The lack of references offers no great obstacle for the student, but references might be desirable to teachers and others who wish to examine the original papers themselves.—  
GEORGE F. WEBER, University of Florida, Gainesville, Florida.

DEVRIES, LOUIS. *German-English Science Dictionary*. 473 p. \$3.00. McGraw-Hill Book Co., Inc. (New York). 1939.

The German-English Science Dictionary, compiled by members of the science faculty of Iowa State College under the editorship of Dr. DeVries, will be welcomed by

all who have to read scientific German in the original and who often have only a passing knowledge of the language. The book contains not only scientific terms but also a wide selection of common words, a fact that endeared Patterson's Chemical Dictionary to its many users. The book is well edited and, notwithstanding its size and variety of subject matter covered, is being sold at a price that should bring it within reach of any graduate student. The compilers succeeded well in bringing together terms found in the various German-English scientific dictionaries of earlier publication date.

It is regrettable, however, that so few new words of the many encountered in recent scientific contributions have been added. For example, under "Vegetation," the editor lists only 7 compound nouns, while the reviewer has in his own footnotes to Biological Equivalents 21 additional words selected from current publications, each of which is not directly translatable but must be expressed by its proper equivalent. This is equally true of the word "Gesellschaft," under which at least 12 new words could be added to the listed 7. Such instances are numerous throughout the book.

The reviewer hopes that in future editions of this volume these omissions will be filled in.—ERNST AETSCHEWAGER.

FUNDAMENTAL STUDIES OF THE STRIPE SMUT OF  
GRASSES (*USTILAGO STRIAEFORMIS*) IN THE  
PACIFIC NORTHWEST<sup>1</sup>

GEORGE W. FISCHER

(Accepted for publication August 30, 1939)

INTRODUCTION

Name of the Disease

The smut of various grasses, caused by *Ustilago striaeformis* (Westd.) Niessl., has been known by such common names as timothy smut, stripe smut, leaf smut, striped smut, and others, the last two names being more widely used. Davis (11) suggested that the disease should be known as striped smut of grasses, rather than timothy smut or leaf smut, because it has been shown that the organism is not confined to timothy, nor is its development restricted to the leaves of the plants infected, but is systemic and may sporulate in culms, leaf sheaths, and floral parts. The writer prefers the name stripe smut because it is more euphonious and is in some respects analogous to the name stripe rust, caused by *Puccinia glumarum* (Schmidt.) Eriks. and Henn.

Economic Importance

The effect of stripe smut on the host makes it a very destructive disease. The rupture of the long sori in the leaves results in the shredding and death of these organs, thereby materially weakening the plant and predisposing it to other sinister factors in its environment. This condition is accentuated by the fact that the leaf sheaths, culms, and floral parts are often attacked. Thus it is obvious that an infestation of stripe smut is a very important factor in the cultivation of grasses for seed, hay, pasturage, and even for lawns and putting greens. Clinton (6) reported substantial losses caused by stripe smut in fields of timothy (*Phleum pratense* L.) and redtop (*Agrostis alba* L.) in Illinois. In one locality, where the latter grass was being grown for seed, Clinton found fully 30 per cent of the plants infected. The grower complained that the seed yield had been reduced to as low as 23 per cent of the normal yield. Osner (29), in making a survey of the extent of stripe smut of timothy in 9 counties of New York, found the disease more or less abundant in every field. In one field he reported over 50 per cent of the plants infected, and estimated that this represented about 30 per cent loss of hay. Osner further pointed out that if the timothy had been grown for seed the loss would have been greater. Pammel *et al.* (32) and Pammel (30, 31) reported considerable loss in the timothy fields on the Iowa State College farm. Numerous other reports could be cited,

<sup>1</sup> Grass-disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Section of Conservation Nurseries, and the Divisions of Plant Pathology and Agronomy of the Agricultural Experiment Station, State College of Washington.

which indicate that the disease is quite widespread from coast to coast. Davis (9) stated that stripe smut is principally confined to the humid central portion of the north temperate zone between the north parallels of 30 and 50 degrees.

#### Host Range

The host range of *Ustilago striaeformis* is quite extensive. Osner (29) listed 38 species and varieties of grasses, including both European and American hosts. Davis (9) stated that this species occurs on about 40 species of the Gramineae, Liro (28) listed over 30 species and varieties, while Seymour (35) in 1929 reported 20 species and varieties from North America. Considering that a number of species have been added since that time by various investigators, and that Seymour's report is not complete to 1929, it seemed probable that over 30 species and varieties of grasses serve as hosts for *U. striaciformis* in North America. In reviewing the available literature on the subject the following list of hosts for North America has been compiled, including a few new hosts established by inoculation experiments described in this paper. *Agropyron caninum* (L.) Beauv.,<sup>2</sup> (17),<sup>3</sup> *A. cristatum* (L.) Gaertn. (17), *A. inerme* (Scribn. and Smith) Rydb. (17), *A. pauciflorum* (Schwein.) Hitchc. (17), *A. repens* (L.) Beauv. (34), *A. semicostatum* (Steud.) Nees,<sup>4</sup> *A. smithii* Rydb.,<sup>4</sup> *A. spicatum* (Pursh) Scribn. and Smith (17), *A. subsecundum* (Link) Hitchc.,<sup>4</sup> *Agrostis alba* L. (35) (under *A. palustris* Huds.), *A. exarata* Trin. (35), *A. perennans* (Walt.) Tuckerm. (39), *A. palustris* Huds. (39), *A. tenuis* Sibth. (24) (as *A. alba vulgaris* Coss. and Dur.), *Amnophila arenaria* (L.) Link (7), *A. breviligulata* Fern. (35), *Bachmannia syzigachne* (Steud.) Fern. (34), *Bromus commutatus* Schrad. (39), *Calamagrostis canadensis* (Michx.) Beauv. (34), *C. pickeringii* A. Gray (35), *Dactylis glomerata* L. (35), *Elymus canadensis* L. (35), *E. canadensis* var. *robustus* (Scribn. and Smith) Mackenz. and Bush.,<sup>4</sup> *E. glaucus* Buckl. (35), *E. sibiricus* L.,<sup>4</sup> *E. virginicus* L. (35), *Festuca obtusa* Spreng. (7) (as *F. nutans*), *F. ovina* L. (35), *Holcus lanatus* L. (36), *Hordeum brevisubulatum* Trin.,<sup>4</sup> *H. jubatum* L.,<sup>4</sup> *H. jubatum* var. *caespitosum* (Scribn.) Hitchc.,<sup>4</sup> *H. nodosum* L.,<sup>4</sup> *Hystrix patula* Moench (25), *Lolium perenne* L. (36), *Phleum pratense* L. (35), *Poa annua* L. (35), *P. compressa* L. (35), *P. languida* Hitchc. (35) (as *P. debilis* Torr.), *P. pratensis* L. (35), *Sitanion hansenii* (Scribn.) J. G. Smith (18), *S. hystrix* (Nutt.) J. G. Smith (7) (as *S. longifolium* J. G. Smith).

#### REVIEW OF LITERATURE

From the contributions of previous investigators of *Ustilago striaeformis* and the smut of grasses induced by it, the following points in the life history and general biology of the pathogen may be summarized:

<sup>2</sup> Where possible Hitchcock (21) has been followed in attempting to determine the correct names for the grasses mentioned in this paper.

<sup>3</sup> Numbers in parentheses refer to literature cited.

<sup>4</sup> Hitherto unreported host, based upon data presented in this paper.

1. Spore germination studies have proved conclusively that the stripe-smut organism is a species of *Ustilago*, and should be called, therefore, *U. striiformis* (6, 9, 29).
2. Spore germination is similar to that reported for *Ustilago nuda* (Jens.) Rostr., *U. tritici* (Pers.) Jens., *U. longissima* (Sow.) Tul., and *U. violacea* (Pers.) Roussel (9).
3. The smut spores of *Ustilago striiformis* are resting spores, and an after-ripening period averaging 240 days in a damp atmosphere at about 20° C. (265 days in field) is prerequisite to successful germination (9).
4. After-ripened spores did not germinate on a variety of substrata unless sufficient moisture was present to float the spores or cover them with a film of solution (9).
5. Inoculations and cytological evidence have demonstrated that, as concerns stripe smut on timothy, seedling infection commonly occurs and is not originated by a dormant mycelium in the embryo (10).
6. At least 4 physiologic races have been differentiated, one restricted to each of the following hosts: *Phleum pratense*, *Agrostis alba*, *Poa pratensis* and *P. annua* (12). *Ustilago clintoniana* (12) and several other species (28) very closely allied to *U. striiformis* may be only physiologic races of the latter species on other hosts.
7. To date all attempts to culture *Ustilago striiformis* have been entirely unsuccessful (9, 10, 24).

#### MATERIALS AND METHODS

In this paper the contributions to the life history and pathogenicity of *Ustilago striiformis* are based upon experiments with two collections of this smut fungus. One collection (herein designated as collection L-A) was made in August, 1936, from several plants in the same row of slender wheat-grass, *Agropyron pauciflorum*, in the Soil Conservation Nurseries, Pullman, Wash. The other collection (collection L-B) came from *Elymus glaucus*, July, 1937, also in the Soil Conservation Nurseries at Pullman.

All inoculations, made with sporidia or with spores, were made by a slight modification of the partial-vacuum method developed by Zade (38), and Haarring (19), and found by Allison (1) and others so effective in studies of smuts of oats and barley. The modification consisted in either planting the inoculated seeds very soon after processing without any drying, or, if the seeds had to be stored before planting, they were dried immediately after processing and were stored dry at room temperature. With either spores or sporidia it seemed to make little if any difference whether the seed was planted immediately after processing or stored dry at room temperature or at 5° C. The procedure followed by Haarring (18), whereby the processed seed was dried for 24 hours, incubated 20 hours in a moisture-saturated atmosphere, and then dried again before sowing, was not tried, since it entailed unnecessary handling of the seed. Allison, after the seed had been processed and then dried for 12 hours, tried two modifications of

Haarring's procedure: (1) after 24 hours' incubation in a water-saturated atmosphere the seed was sown while still moist; (2) seed was stored dry at 2° C. for 24 hours before sowing. He found that the two modified methods gave equally excellent results, and finally adopted the second because of its simplicity. In the present studies not even refrigerated storage was found necessary.

When chlamydospores were used for inoculum the desired suspension was obtained by placing infected leaves and other parts in a beaker or flask with a small amount of water and macerating the material so as to liberate the spores. Water was then added to bring the suspension to the proper dilution after which the bits of leaves and other debris were strained off. The seed was inoculated in small (15 cc.) vials each containing about 5 cc. of the suspension.

When monosporidial lines of opposite sex were used for inoculum the technique of preparing this was somewhat different from that used by Allison (1), who grew the lines in potato-dextrose solution and used the resulting suspension of sporidia in the nutrient solution for inoculum. In the present studies the sporidia were transferred from the agar to water or physiological salt solution, and the resulting suspension was used for inoculum. Copious production of fresh sporidia was obtained in 2 days by making smear cultures on plates of a special agar medium, described below, containing 8 per cent dextrose, 4 per cent malt extract, 1 per cent peptone, and 2 per cent agar. The monosporidial lines of opposite sex were thus cultured singly on the agar, but were brought together in essentially equal quantities in the inoculum suspension.

The monosporidial lines of *Ustilago striiformis* were obtained by isolating single sporidia from germinating chlamydospores with the aid of a Chamber's micro-manipulator, following the method described by Hanna (18).

#### EXPERIMENTAL RESULTS

##### Life History Studies

1. *Spore Germination.* According to Davis (10) an after-ripening period of 240–250 days in a moist chamber at room temperature is prerequisite to spore germination in *Ustilago striiformis*, which probably explains the very low germination percentages obtained by Osner (29), Clinton (6), Horsfall (23), Duggar (15), and others. However, some investigators apparently have not experienced such difficulty in germinating the spores of *U. striiformis*. Liro (28) presents the results of a series of inoculation experiments with stripe smut on various grasses and makes no mention of any prerequisite to successful spore germination. He obtained infection merely by mixing dry seeds and spores together. However, it must be considered that Liro worked with collections of stripe smut from other grasses than those employed in Davis' experiments. On the other hand, Davis (8) stated, concerning collections of *U. striiformis* on *Poa*

*pratensis* and *P. debilis* (*P. languida*), that fresh spores collected in May germinated readily in water. Pammel (33) mentioned that spores of *Tilletia striiformis* (Westd.) Magnus (*U. striiformis*) from *Phleum pratense* and *Poa pratensis* germinated readily. He did not state the medium.

In July, 1938, the writer received a collection of *Ustilago striiformis* on *Poa pratensis* from H. W. Johnson, Bureau of Plant Industry, who had obtained it from the Forage Crops greenhouse at Arlington, Virginia, May 5, 1938. On July 25 the spores were tested for viability and, after about 36 hours, 85 per cent of them had germinated on potato-dextrose agar. Subsequent tests gave gradually lower percentages until, finally, the last test, on October 10, 1938, showed no germination at all. Thus, this collection of *Ustilago striiformis* exhibited viable spores, with a high percentage of germination, merely after dry storage at room temperature.

A collection of *Ustilago striiformis* on *Agropyron pauciflorum* (collection L-A mentioned above) made in August, 1936, was tested for spore viability as soon as it could be brought into the laboratory, but less than 5 per cent germination was observed. Another test late in September showed about 25 per cent viable spores. This collection was not tested further until the following spring, when less than 1 per cent germination was observed. Several of the infected plants were removed to the greenhouse in the fall of 1936, where they continued to produce smutted leaves, and later, culms and spikes. In March, 1937, a quantity of infected leaves was removed from these potted plants and samples of the spores were tested for viability. Even in cases where spores had been removed from fresh, unruptured sori, as high as 90 per cent germination was observed. It should be mentioned that the smutted leaves dried in the laboratory for a few days before the spores were tested. This spore material was used in spring inoculation experiments of 1937.

In July, 1937, *Ustilago striiformis* was found on *Elymus glaucus* (collection L-B, mentioned above). At that time tests showed over 60 per cent spore germination. Subsequent tests every several weeks showed a gradual decrease in percentage of germination, but this was about 20 per cent, even as late as March 1, 1938, when monosporidial cultures were obtained from germinating chlamydospores. In a later test, May 26, 1938, there was less than 1 per cent germination.

Another collection, made in August, 1938, on *Elymus canadensis* in the Pullman, Wash., Soil Conservation Nurseries, was not tested for spore viability until March 13, 1939, when 50 per cent spore germination was obtained on malt extract-dextrose-peptone agar.

In contrast to the collections described in which good germination was secured without any after-ripening process, are several others from *Poa pratensis*, *Phleum pratense*, *Agrostis alba*, and *Holcus lanatus*, which did not germinate over 2 per cent over a period of 5 months.

All of the above-described collections had been stored in envelopes or packets under conditions of room temperature and humidity, the latter

usually running rather low in the dry-land areas of the Pacific Northwest. It is seen from the spore germination data of these collections that for some reason the after-ripening period, under moist conditions described by Davis (9) as prerequisite to spore germination, does not apply to all collections of *Ustilago striaeformis*, especially the race on *Agropyron* and *Elymus* in the Pacific Northwest. It is further seen that the collections of *U. striaeformis* on *Agropyron* and *Elymus* in the Pacific Northwest have a greater period of germinability than those studied by Davis (9). He found that approximately 75 days represented the period of germinability for the smut from timothy, orchard grass, and redtop, and 120 days for stripe smut on June grass (Kentucky blue grass). The writer's *Agropyron* and *Elymus* collections of *U. striaeformis* indicated a period of germinability of several months.

The process of germination in the collections of *Ustilago striaeformis* on *Agropyron pauciflorum* (col. L-A) and on *Elymus glaucus* (col. L-B) has been studied and appears to differ from that reported by Davis (9) for collections on *Agrostis alba*, *Dactylis glomerata*, *Phleum pratense*, and *Poa pratense*, and by Osner (29) for *U. striaeformis* on *Phleum pratense*. These investigators found the process to be the same in the collections from all 4 hosts. A single elongate unicellular promycelium was extruded from a rift in the spore wall. Sometimes 1 or 2 septa were formed. Short branches usually were developed on the promycelium and were considered to be lateral sporidia, although they were not abstricted. In a few cases Davis observed fusions between these "sporidia." No typical sporidia were observed, and Davis was of the opinion that they are seldom, if ever, produced. In no case did he obtain any saprophytic development from the germinating spores, even when they had been under observation for 60 days.

In the present studies it was observed that, after 24-36 hours under optimum conditions for germination, there emerges from the spore 2 or 3 thick germ tubes (Fig. 1, A and B). These rapidly elongate, and develop cross walls and branches (Fig. 1, C and D), and soon begin to bud off elliptical sporidia. By the third day numerous sporidia usually have been produced (Fig. 1, E) and accumulate in masses, so that around each germinated spore there develops a vigorous saprophytic colony of mycelium and sporidia. This type of germination occurs on potato-dextrose agar, or malt extract-dextrose-peptone agar, but on low nutrient agars or on plain agar sporidial formation is more or less inhibited. In such cases fusions between promycelial branches or segments soon occur and these give rise to long, vigorous, aerial infection hyphae. This is thought to be comparable to what Davis (9) observed and described as, "In three cases primary sporidia fused, the contents of one passed into the other and formed a conidium which developed a mycelial thread."

No observations by the writer of germinating spores of *Ustilago striaeformis* on a variety of media have yielded anything comparable to what Davis (9) observed and described as primary sporidia emerging directly

from the spore and budding. At other times, according to Davis, the "primary sporidia" remained within the spore. The sporidia observed in the present experiments were larger, more typical of sporidia in general, and

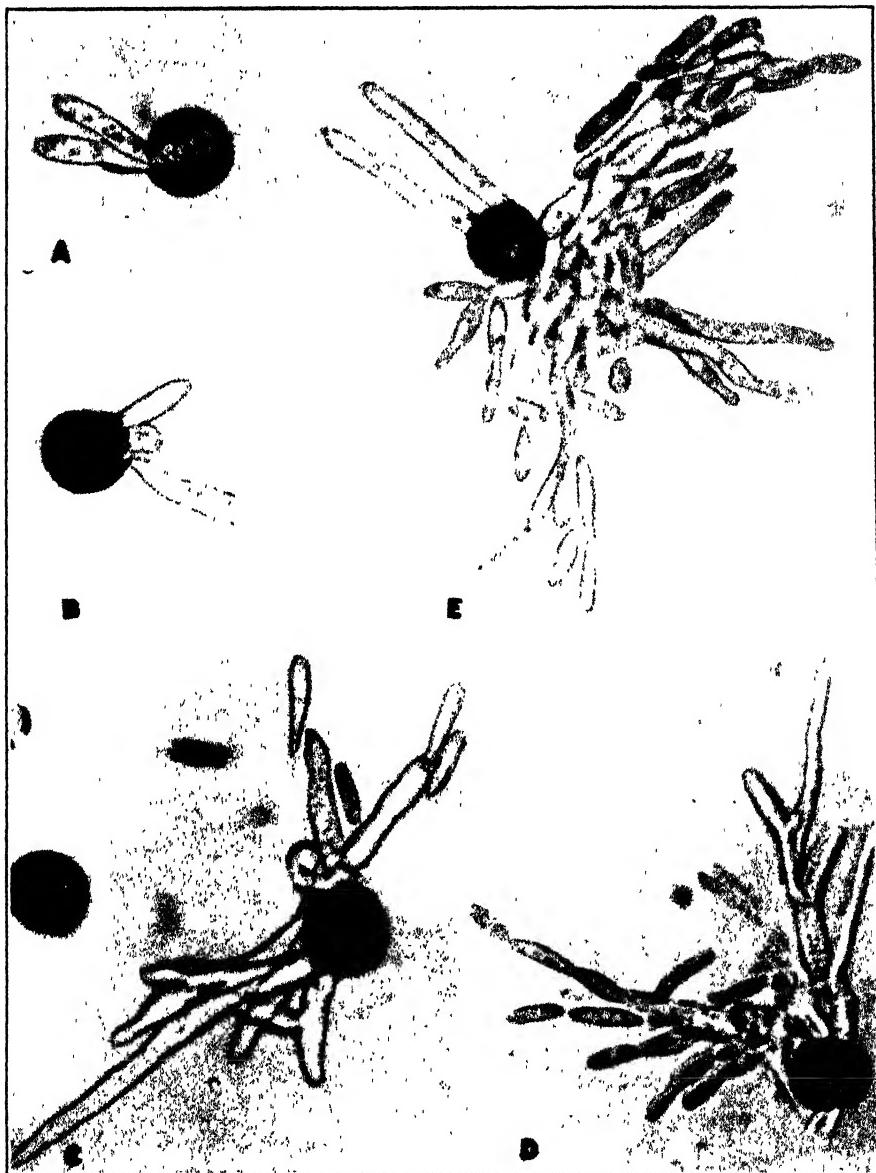


FIG. 1. Stages in spore germination in *Ustilago striaeformis*: A, B, after 36 hrs. on potato-dextrose agar; C, D, after 48 hrs.; E, after 60 hrs. Retouched enlargements of photomicrographs.  $\times$  approximately 1000.

were abstricted from the promycelial branches or segments. They were able to develop rapidly into large saprophytic colonies, an ability which heretofore has been entirely unknown in *U. striaeformis*.

**2. The Sexual Nature of the Sporidia.** Sometimes on nutrient agar, *in situ*, but especially when removed to plain, non-nutrient agar, the sporidia fuse in pairs with surprising rapidity. This fusion is accomplished when one (active) sporidium sends out a narrow fusion tube toward another (passive) sporidium nearby (Fig. 2, A) and fuses with that sporidium when contact has been made (Fig. 2, C-G). Occasionally, the juxtaposition of sporidia is such that 2 will try to fuse with the same sporidium, as seen in figure 2, B. Quite often 2 fused sporidia will be observed connected by a very long fusion tube, as shown in figure 2, H. Whether this represents the elongation of the fusion tube before fusion (in order to reach a sporidium some distance away) or an elongation after fusion, as Holton (22) observed in *U. avenae* and *U. levis*, has not been determined.

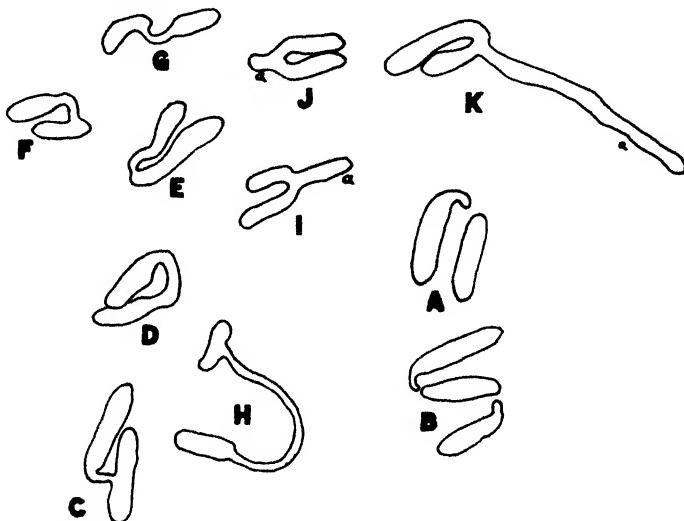


FIG. 2. Stages in the copulation of sporidia of *Ustilago striaeformis* and the subsequent development of infection hyphae. A and B. Development of fusion or copulation tube. C-H. Appearance of pairs of fused sporidia. I-K. Development of infection hypha, a. Drawn with the aid of the camera lucida.  $\times$  about 1100.

Shortly after fusion (2-4 hours), from one or the other of the fused pair, or from the fusion tube connecting them, a papilla appears (Fig. 2, I, a and J, a), which rapidly develops (Fig. 2, K, a) into a long vigorous aerial hypha. This is the "Suchfäden" described by Bauch (3, 4, 5). Within 12 hours after sporidia of opposite sex have been mixed on plain agar these "Suchfäden" or infection-hyphae have been produced in abundance and lend a cottony or whitish cast to the whole surface. Thus the well-known "Bauch test" (3, 4) for detecting sporidial lines of opposite sex is readily applicable to *Ustilago striaeformis*. In fact the fusion of sporidia and the subsequent production of "Suchfäden" or infection hyphae is very similar to that described for *Ustilago avenae* (23), *U. levis* (13, 23), *U. hordei* (1, 33), *U. bromivora* (Tul.) Fisch. de Waldh. (3), *U. violacea* (2, 27), *U. nigra* Tapke (*U. medians* Biedenk. (1)) and others.

The readiness with which the sporidia of the writer's cultures of *Ustilago striiformis* fuse and develop infection hyphae indicated their sexual nature, and it was decided to investigate this further. Some preliminary studies of monosporidial lines will be reported here.

On March 1, 1938, from a small colony that had developed around a single germinated spore of collection L-B of *Ustilago striiformis* (from *Elymus glaucus*), 15 sporidia were isolated and allowed to bud and develop into monosporidial colonies. Only 9 sporidia survived the isolating process, but these developed rapidly into colonies that were later transferred to test tubes of potato-dextrose agar. By March 10, 1938, each culture had grown to sufficient size to afford an abundance of material for inter-mating of the cultures. Accordingly, a small amount of each culture was mixed, in turn, with an equal amount of each of the other cultures on plain agar. Within 12 hours or even less it was possible to tell at a glance under the low power of the microscope where fusions had taken place. In fact, the whitish cast visible to the naked eye indicated where fusions and the production of infection hyphae had taken place. The results of these matings are shown in table 1.

TABLE 1.—Reactions of nine monosporidial cultures of *Ustilago striiformis* (L-B)<sup>a</sup> from the same germinating chlamydospore, when mated with each other on plain agar

		Designation of cultures								
		11	12	13	14	15	16	17	18	19
11	- <sup>b</sup>	-	-	-	+	-	+	+	-	+
12	-	-	-	-	+	-	+	-	-	+
13	-	-	-	+	-	-	-	-	-	+
14	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	+	-	-	-	+
16	-	-	-	-	-	-	-	+	-	-
17	-	-	-	-	-	-	-	+	-	-
18	-	-	-	-	-	-	-	-	-	+
19	-	-	-	-	-	-	-	-	-	-

<sup>a</sup> Collection L-B is from *Elymus glaucus*.

<sup>b</sup> - = abundant fusions and infection hyphae.

<sup>b</sup> Legend: - = no action.

On the basis of the reactions shown in table 1 it is seen that of the 9 cultures, 5 were of 1 sex group and 4 of another. By arbitrarily designating the first culture (No. 11) as the others are segregated for sex as follows:

Culture No. 11	+	+	+	-	+	-	-	+	-
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From these results it seems that only 2 sex groups are represented in *Ustilago striiformis* and that these probably are segregated on a 1:1 basis.

3. "Hybridization" with Other *Ustilago* spp. Kniep (27) considered hybridization as having been effected between species of smut fungi when their sporidia had been made to fuse. He obtained fusions between the

sporidia of several *Ustilago* spp. but did not attempt to obtain F<sub>1</sub> chlamydospores from these combinations by inoculating the fused sporidia into a probable host. Dickinson (14) obtained infection (as evidenced by histological examination of inoculated oat seedlings) from combinations of sporidia of opposite sex of *U. levis* and *U. hordei*, but did not state that any sporulation occurred in the host. Since neither Kniep nor Dickinson obtained F<sub>1</sub> chlamydospores from their "hybrids," and since Allison (1) has shown that, at least in the barley smuts, soon after the fusion of two sporidia (of different as well as of same species) merely the dikaryophase is initiated by association of the two nuclei in one sporidium and later in the infection hypha, the fusing of sporidia cannot be considered true hybridization. It is fairly well established that in the smut fungi this dikaryophase persists throughout the development of the mycelium in the host plant up to the time of sporulation, and that the true diploid condition is initiated at the time of fusion of the two nuclei in the young spore. True hybridization occurs, then, when interspecific combinations of monosporidial lines of opposite sex result in sporulation on some host. Such hybrids have been produced between *U. avenae* and *U. levis* (22), *U. hordei* and *U. nigra* (1), *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter (34), *S. sorghi* and *Sorosporium reilianum* (Kühn) McAlpine (37), and other species.

In the present studies 4 monosporidial lines of *Ustilago striaeformis*, L-B, (2 of each sex group) were mated with 12 pedigree monosporidial lines of *U. bullata* (1 of each of 2 sex groups from 6 collections) on plain agar. The results are recorded in table 2.

From the data shown in table 2 it is readily seen that: (1) The sporidia of *Ustilago striaeformis* are highly compatible with those of *U. bullata*; (2) certain combinations are far more productive of infection hyphae than are others; and (3) in every case the greater number of infection hyphae involved the same sex group of *U. striaeformis*. Thus, it was cultures L-B 11 and 12 that produced the abundance of infection hyphae when combined with cultures of opposite sex of *U. bullata*, although the other cultures of *U. striaeformis*, L-B 17 and 19, entered just as readily into sporidial fusions with the cultures of opposite sex of *U. bullata*. This situation is somewhat similar to that described by Bauch (4) who discovered that *Sphacelotheca schweinfurthiana* (v. Thüm.) Sacc. exhibited 2 different types of reaction between the monosporidial lines of opposite sex; in one case ("W-Reaktion") no further development of the fused sporidia took place, whereas, in the other ("S-Reaktion"), binucleate infection hyphae developed from the fused sporidia in the normal manner. Bauch's results, however, were obtained from intraspecific mating experiments, whereas those here described apply to interspecific matings. Within either of the species concerned, *U. striaeformis* and *U. bullata*, such reactional differences have not been observed. These differences are thought by the writer to represent differences in the compatibility between monosporidial lines of opposite sex of 2 species

TABLE 2.—Reactions of 4 monosporidial cultures of *Ustilago striaeformis* with 12 pedigree monosporidial cultures of *Ustilago bullata* when mixed on plain agar

Ustilago bullata	Pedigreed monosporidial cultures	<i>U. striaeformis</i> , L-B, <sup>b</sup> Monosporidial cultures			
		11	12	17	19
<i>U. bullata</i>	N-A 111	++++*	++++	-	-
" "	" 112	-	-	+	+
" "	N-J 51	-	-	+	+
" "	" 52	++	++++	-	-
" "	R-A 71	-	-	+	+
" "	" 72	++	++	-	-
" "	R-G 51	-	-	++	++
" "	" 53	+++	++++	-	-
" "	M-C 121	-	-	+	+
" "	" 123	++	++	-	-
" "	M-L 61	++	+++	-	-
" "	" 63	-	-	+	+

\*Legend: ++++ = abundance of both fusions and infection hyphae.

+++ = " " " fusions but less of infection hyphae.

++ = " " " , with few infection hyphae.

+= " " " , " very few or no infection hyphae.

- = neither fusions nor infection hyphae.

<sup>b</sup> *U. striaeformis* L-B = collection from *Elymus glaucus*; *U. bullata* N-A = collection from *Agropyron pauciflorum*, N-J = col. from *E. canadensis*; R-A = *ibid.* from *Hordeum nodosum*; R-G = *ibid.* from *Sitanion jubatum* J. G. Smith; M-C = *ibid.* from *Bromus marginatus* Nees.; M-L = *ibid.* from *Bromus anomalus* Rupr. M-C and M-L have earlier been known as *U. bromivora* (Tul.) Fisch. von Waldh. (16). The arabic numerals appended to the collection symbols indicate the pedigree of the monosporidial cultures. In every case the last digit indicates the origin of the culture with reference to the position, on the promycelium, of the isolated sporidium from which the culture originated. The remaining digit or digits refer to the number given the chlamydospore from whose promycelium the sporidia were isolated. The numbering of the chlamydospores always begins with the digit 5, so as to avoid confusion with sporidium numbers that ordinarily range from 1 to 4. Arbitrarily, the sporidium from the distal cell of the promycelium is designated as No. 1, and the numbering proceeds toward the spore. (Thus, monosporidial culture M-C 123 arose from the third sporidium on the promycelium of chlamydospore No. 12). This method of indicating pedigree could not be applied to *U. striaeformis* because of the indefinite position of the sporidia on the branched promycelium.

of smut fungi, due to the association, in the copulating sporidia, of 2 nuclei representing the 2 different species. In one case the 2 nuclei are compatible and an infection hypha results; in the other case the association is incompatible and usually no further development takes place. However, this fundamental difference between the nuclei must be independent of sex potential; otherwise, presumably, sporidia of opposite sex, but having incompatible nuclei, would not even fuse.

The profuse development of infection hyphae resulting from sporidial fusions between certain monosporidial lines of opposite sex of *Ustilago striaeformis* and *U. bullata*, as compared with the absence of such infection hyphae in other combinations of opposite sex of the same species, suggests that the former type might be expected to represent sufficient compatibility to result in the development of mycelium and  $F_1$  chlamydospores when applied to some common host. Likewise, the sporidial fusions wherein very few or no infection hyphae result might not be expected to result in such infection and spore production. Experiments are in progress to test out

these possibilities on *Agropyron pauciflorum*, *Elymus canadensis* and *E. sibiricus*, which are common hosts for both species of smut fungi.

Cultures L-B 11, 12, 17, and 19 of *Ustilago striiformis* also were mated with monosporidial cultures of *Ustilago nigra* and *U. hordei*, but, although in some cases rather numerous sporidial fusions resulted, a very low degree of compatibility was indicated as compared with the combinations with *U. bullata*.

4. *Behavior of Ustilago striiformis in Culture on Agar Media.* Although earlier investigators have reported that *Ustilago striiformis* is incapable of development on artificial media, the writer has found that the race of this species, which occurs on *Agropyron* and *Elymus* in the Northwest, is one of the most easily cultured and most rapidly growing smut fungi in his experience.

Shortly after obtaining the 9 monosporidial cultures of opposite sex, as described above, it was decided to try a culture of each of the 2 sex groups on different agar media. For a preliminary experiment 4 agar media were finally selected: (1) raisin agar, containing an infusion from 100 grams raisins per liter, with .1 per cent peptone added; (2) potato-dextrose agar, containing 200 grams potatoes and 20 grams dextrose per liter; (3) "P. D. A. +," a modification of potato-dextrose agar, containing 1.5 per cent malt extract and .1 per cent peptone per liter; and (4) pea agar, containing an infusion from 300 grams dried peas, 2 per cent dextrose, 1.5 per cent malt extract, and .1 per cent peptone per liter. Duplicate 50 mm. Petri dishes of these media were inoculated and incubated at room temperature (20-22° C.) for four weeks. The growth response of the two cultures is illustrated in figure 3. The growth on the raisin agar was rather weak, but was good on the other three media. With both sexes the best growth was obtained on the modified potato-dextrose agar.

Since the agar containing potatoes, dextrose, malt extract, and peptone proved to be the best in the above experiment, an attempt was made to determine the optimum proportion of these ingredients. Accordingly, 25 different combinations of these, all in 2 per cent agar, were prepared and poured into 50 mm. Petri dishes (Table 3), and represent only third of the possible combinations at the gradations shown. In conjunction with a similar experiment with other grass smut fungi, these plates were inoculated in duplicate with one monosporidial culture of *Ustilago striiformis* (L-B 17). They were incubated at room temperature for 2 weeks, at the end of which time the diameter in mm. of each colony was recorded. The data are presented in table 3 and the growth response is shown in figure 4.

The results of the above experiment indicate a rather wide tolerance in *Ustilago striiformis* of high concentrations of the nutrients used. It is surprising that growth was obtained on agar No. 25, containing 40 per cent dextrose, as is the tolerance to 35 per cent dextrose in agars No. 20 and 24. One of the agars resulting in the largest colonies is No. 15, containing 25 per cent dextrose; but, on the other hand, some of the other agars just as

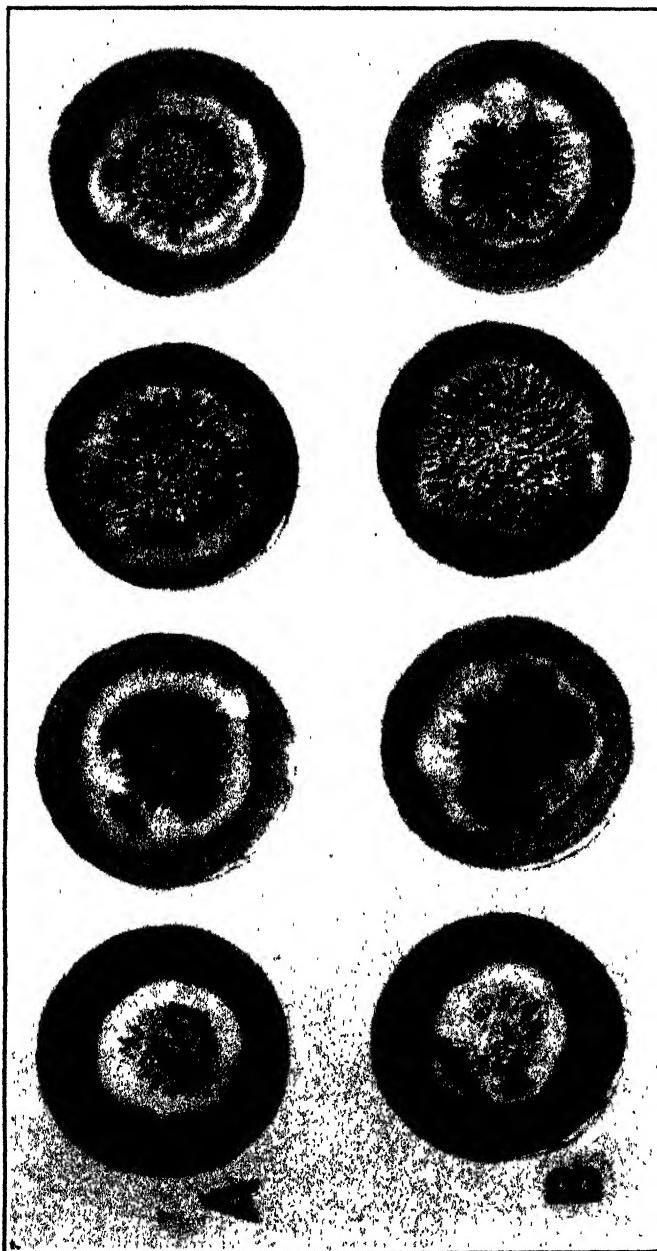


FIG. 3. Growth of *Ustilago striaeformis* (two cultures of opposite sex) on agar media. A. Culture L-B 11 on (left to right) raisin agar, potato-dextrose agar, potato-dextrose malt-extract agar, and pea-infusion agar. B. Culture L-B 19 on the same media. Four-weeks growth in 50-mm. Petri dishes, at room temperature.

TABLE 3.—Summary of the size of colonies produced on 25 combinations of potato decoction, dextrose, malt extract, and peptone in 2 per cent agar, by *Ustilago striiformis* (L-B). Two weeks' growth at room temperature

Agar No.	Dextrose per cent	Composition of the agars			L-B 17
		Potatoes grams	Malt extract per cent	Peptone per cent	Diameter of colony mm
1	0	400	20	0.2	15
2	1	300	15	0.3	15
3	2	200	10	0.5	24
4	4	100	6	0.75	24
5	8	0	4	1.00	26
6	1	500	15	0.15	16
7	2	400	10	0.2	13
8	4	300	6	0.3	15
9	8	200	4	0.5	16
10	15	100	2	0.75	20
11	2	600	10	0.1	10
12	4	500	6	0.15	19
13	8	400	4	0.2	23
14	15	300	2	0.3	14
15	25	200	1	0.5	27
16	4	700	6	0.05	18
17	8	600	4	0.1	27
18	15	500	2	0.15	24
19	25	400	1	0.2	20
20	35	300	0.5	0.3	16
21	8	800	4	0.0	27
22	15	700	2	0.05	25
23	25	600	1	0.1	20
24	35	500	0.5	0.15	13
25	40	400	0	0.2	6

good, at least from the standpoint of the size of colony produced, numbers 3, 4, 5, 17, 18, 21, 22, contain from 2-15 per cent dextrose.

It would appear from the data that different amounts of peptone and potato cause less difference in growth response, from the standpoint of size of colony, than do the dextrose and malt extract. However, the experiment indicates that, while no one combination of the 25 tested is undoubtedly optimum, certain proportions of potato, dextrose, malt extract, and peptone are decidedly better than others for the production of vigorous colonies of a good sporidial consistency. In general, agar No. 5, containing 8 per cent dextrose, 4 per cent malt extract, and 1 per cent peptone, represents one of the best if not the best combination tried. This formula is being used at the present time for all of the writer's cultures of *Ustilago striiformis*, as well as for other smut fungi.

#### Inoculation Experiments

Inoculation experiments with *Ustilago striiformis* have been performed by Davis (10, 11, 12), Osner (29), and Liro (28), although the work by Liro was done under other species (biologic species) names. Davis inoculated seedlings of *Agrostis alba*, *Dactylis glomerata*, *Phleum pratense*, and *Poa* spp. Osner inoculated *Phleum pratense* and obtained very inconclusive

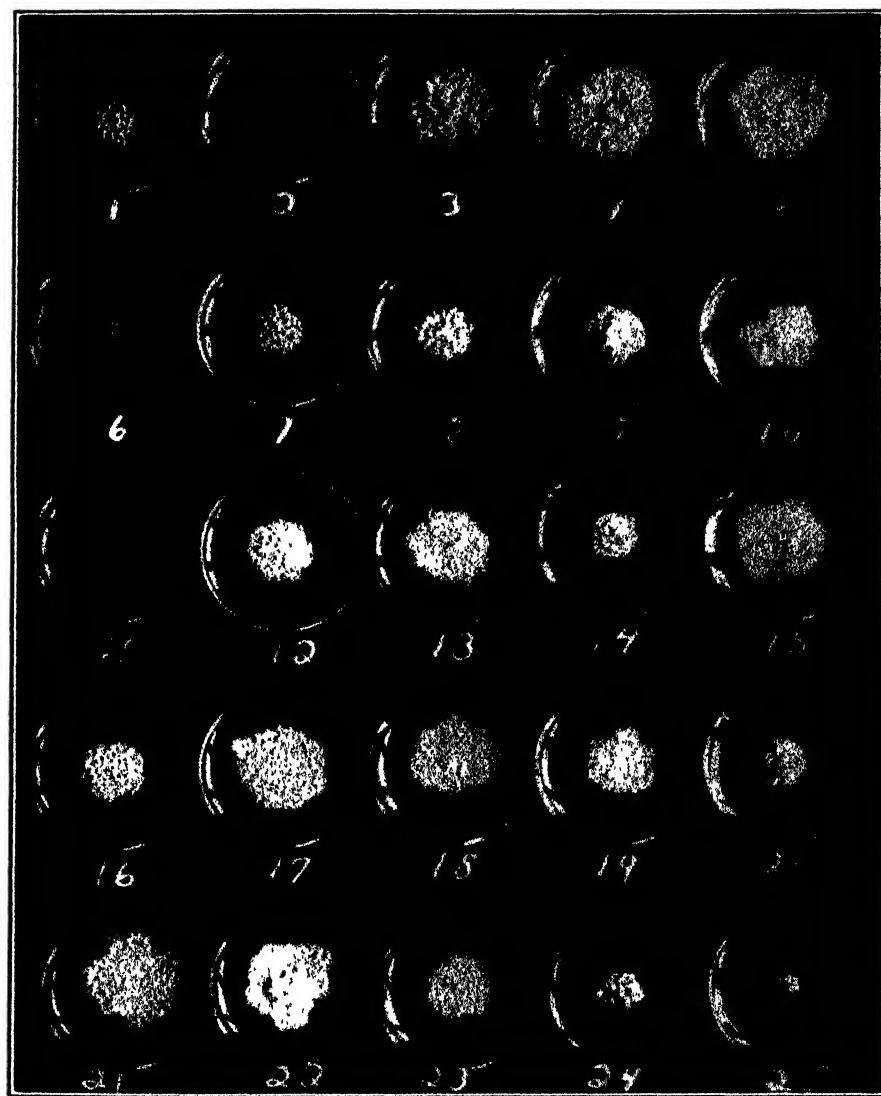


FIG. 4. Growth of *Ustilago striiformis* on 25 different combinations of potato decoction, dextrose, malt extract, and peptone in 2 per cent agar. Compare with Table 3. Four-week growth in 50-mm. Petri dishes.

results, it being obvious that his seed already carried the fungus. Davis secured no higher than 60 per cent infection of timothy seedlings by seedling inoculation, and, even then, the hulls had been removed from the seed. By planting timothy seed in soil artificially contaminated with smut spores of *U. striiformis*, he obtained as high as 72 per cent infection. His method of inoculation was quite laborious: the inoculum had to be "after-ripened" under specified conditions of time, temperature, and moisture; the spores would not germinate unless covered with a film of water; and the seeds were (except by soil inoculation) germinated and hulled before inoculation.

Osner (31) believed he had sufficient evidence to prove that blossom infection obtained in *Ustilago striaeformis*, but Davis (11) definitely disproved this. Exhaustive blossom-inoculation experiments gave negative results.

Liro (28) obtained satisfactory results merely by mixing seed and spores together (presumably dry).

1. *Field Experiments in 1937.* Inoculation experiments with *Ustilago striaeformis* by the writer indicate that such studies with this race on *Agropyron*, *Elymus*, etc., in the Northwest involved considerably less complication and trouble than was experienced by Davis in his studies of races on *Agrostis*, *Phleum*, *Poa*, etc. Noting that some plants of *Agropyron pauciflorum* in the greenhouse (transplanted from the field where they had been found infected with stripe smut) were producing sori in abundance somewhat prior to the time of the annual spring (1937) seeding and inoculation experiments, it seemed that here might be some inoculum for such experiments with stripe smut. Spores smeared over potato-dextrose agar germinated about 90 per cent. Considerable inoculum was collected from these greenhouse plants over a period of about a month and very little reduction in percentage of germination was observed during this time. The inoculum was prepared in the form of a spore suspension as already described along with the method of inoculation, under "Materials and Methods."

As a preliminary experiment, 50 seeds of each of the grasses listed in table 4 were inoculated, allowed to dry enough to permit handling, and then sown in 5-foot nursery rows. Within a few weeks after seeding, some of the grasses (*Agropyron subsecundum*, *A. pauciflorum*, et al.) began to show the first symptoms of infection, and others followed sooner or later. The final data from this experiment are present in table 4.

The data recorded in table 4, while based on too few plants per row to be very reliable, did nevertheless indicate the susceptibility of various species of *Agropyron*, *Elymus*, and *Hordeum* to this collection of *Ustilago striaeformis* from *Agropyron pauciflorum*.

2. *Field Experiments in 1938.* In March, 1938, after monosporidial cultures of opposite sex of *Ustilago striaeformis* had been obtained, it was decided to try these as inoculum. Allison (1) recently had reported great success in the use of suspensions of sporidia of opposite sex as inoculum in his studies of *U. hordei* and *U. nigra*. It was thought that if sporidial cultures of the stripe-smut organism could be used for inoculum, one need not be concerned with having a supply of viable chlamydospores for such purposes.

As a preliminary test of the efficacy of suspensions of sporidia of opposite sex as inoculum, approximately 200 seeds of *Agropyron subsecundum* (Acc. No. 13), shown in 1937 to be highly susceptible, were so inoculated, using the partial vacuum method, and sown in rows in the greenhouse. The seed was not allowed to dry before planting. Within 4 weeks after planting the first sori appeared; at the end of 6 weeks they were abundant. Of 164

TABLE 4.—Reaction of *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum* and *Sitanion* spp. to *Ustilago striaeformis* from *Agropyron pauciflorum*.

Grass species	Writer's Acc. No.	No. plants	No. smutted	Percentage infection
<i>Agropyron caninum</i>	282	9	9	100
" <i>cristatum</i>	290	1	1	100
" "	304	— <sup>a</sup>	0	0
" <i>elongatum</i>	299	—	0	0
" <i>inerme</i>	268	1	1	100
" "	270	5	1	20
" <i>pauciflorum</i>	249	—	0	0
" "	288	5	1	20
" "	251	4	2	50
" "	324	—	7	100
" <i>pungens</i>	298	—	0	0
" <i>sibiricum</i>	291	—	0	0
" <i>smithii</i>	285	—	0	0
" <i>subsecundum</i>	13	6	6	100
" "	309	—	0	0
" <i>spicatum</i>	264	—	0	0
" "	265	3	1	33
" <i>repens</i>	297	—	0	0
" <i>trichophorum</i>	296	—	0	0
<i>Bromus ciliatus</i>	247	—	0	0
" <i>marginatus</i>	48	—	0	0
" <i>tertorum</i>	165	—	0	0
<i>Elymus canadensis</i>	128	—	0	0
" <i>glaucus</i>	3	4	4	100
" "	5	3	1	33
" <i>striatus</i>	124	—	0	0
" <i>virginicus</i>	131-35	0	0	0
<i>Festuca elatior</i>	191	—	0	0
<i>Hordeum brevisubulatum</i>	302	5	1	20
<i>Sitanion hystrix</i>	300	—	0	0

<sup>a</sup>—number of plants was not recorded in rows showing no smut.

plants in the rows, 149, or about 90 per cent, showed smut typically and unmistakably. A check row of the same grass contained no smut. This preliminary test indicated that sporidia should be reliable inoculum for field plantings.

In the spring of 1938 (Apr. 16-20) the following species were inoculated with sporidia of opposite sex and seeded in 5½-ft. nursery rows: *Agropyron caninum*, *A. ciliare*, *A. cristatum*, *A. desertorum*, *A. elongatum* (Host) Beauv., *A. inerme*, *A. pauciflorum* (34 collections and selections), *A. pungens*, *A. sibiricum*, *A. smithii*, *A. spicatum*, *A. semicostatum*, *A. subsecundum*, *A. trichophorum* (Link) Richt.; *Agrostis alba*; *Arrhenatherum elatius* (L.) Mert. and Koch; *Bromus ciliatus* L., *B. marginatus* Nees; *Dactylis glomerata*, *Elymus canadensis*, *E. sibiricus*, *E. villosus* Muhl., *E. virginicus* var. *australis* (Scribn. and Ball) Hitchc.; *Holcus lanatus*; *Hordeum brevisubulatum*, *H. bulbosum* L., *H. gussoneanum* Parl., *H. jubatum*, *H. jubatum* var. *caespitosum*, *H. murinum* L.; *Lolium perenne*; *Phleum pratense*; *Poa pratensis*; *Sitanion hystrix*.

In general, good stands resulted from these inoculated seeds. In some rows smut began to appear as early as June 1. Infection counts were de-

layed until about the middle of July when it was noticed that in some of the rows the plants were succumbing to the infection. Row by row, the plants were uprooted and the total number and the number showing smut in each row were recorded (Table 5). The data concerning the 34 selections and

TABLE 5.—*Reaction of Agropyron, Elymus and Hordeum spp. to a collection L-B of Ustilago striiformis from Elymus glaucus*

Species	Writer's Acc. No.	S.C.N. No.	No. plants	No. smutted	Percent- age smut
<i>Agropyron cristatum</i>	29		15	2	13
" "	36		72	11	15
"	290	W. 2413	138	25	18
" <i>semicostatum</i>	377	W. 2903	75	11	15
" <i>subsecundum</i>	309		48	20	42
" "	12		37	5	14
" "	13		30	18	60
" sp.	310		112	2	
<i>Elymus canadensis</i>	341	W. 2389	79	61	77
" <i>sibiricus</i>	335	W. 214	79	51	65
" "	338	W. 225	201	181	90
<i>Hordeum brevisubulatum</i>	302	W. 303	85	8	9
" <i>jubatum</i> var.					
" <i>caespitosum</i>	334		13	12	92
" <i>jubatum</i>	15		33	17	52

collections of slender wheatgrass will be presented and discussed below, under "Varietal reaction of selections and collections of slender wheatgrass to *Ustilago striiformis*."

Of the 33 species and varieties inoculated only 7 showed smut. These are listed in table 5. On the basis of these results *Agropyron pauciflorum*, *A. subsecundum*, *Elymus canadensis*, *E. sibiricus*, *Hordeum jubatum*, and *H. jubatum* var. *caespitosum* are highly susceptible to this collection (L-B) of *Ustilago striiformis* from *Elymus glaucus*. The absence of even a trace of stripe smut on any of the accessions of redtop, timothy, orchard grass, Kentucky blue grass, and velvet grass indicates further proof that the stripe smut on *Agropyron* and *Elymus* in the Northwest is a race very distinct from the races studied by Davis (11, 12) and Osner (29).

3. *Greenhouse Experiments.* The successful inoculation of *Agropyron subsecundum* with *Ustilago striiformis* in the greenhouse, as described above, suggested that possibly studies of the stripe smut of grasses could be carried out in the greenhouse, since it would not be necessary for the grasses to come to maturity, as it is with inoculation experiments with most smuts. If good infection could be obtained from sporidia or spores and a reading obtained within 6 weeks, as the preliminary inoculation of *A. subsecundum* indicated, then greenhouse studies of stripe smut could be pursued with almost as much ease as are those that concern the rusts of cereals and grasses.

On Jan. 21, 1939, 40–50 seeds of each of the following grasses were inoculated with (1) chlamydospores of *Ustilago striiformis* collection L-A (from *Agropyron pauciflorum*), (2) chlamydospores of collection L-B (from

*Elymus glaucus*), and (3) suspensions of sporidia from monosporidial cultures of opposite sex of collection L-B: *Agropyron caninum*, *A. ciliare* Franch., *A. cristatum*, *A. desertorum* (Fisch.) Schult., *A. elongatum*, *A. inerme*, *A. repens*, *A. semicostatum*, *A. sibiricum* (Willd.) Beauv., *A. smithii*, *A. spicatum*, *A. subsecundum*, *A. trichophorum*; *Agrostis alba*; *Arrhenatherum elatius*; *Bromus brachystachys*, *B. brizaeformis* Fisch. and Mey., *B. erectus* Huds., *B. hordeaceous* L., *B. inermis* Leyss., *B. japonicus* Thunb., *B. mollis* L., *B. tectorum* L.; *Dactylis glomerata*; *Elymus canadensis*, *E. canadensis* var. *robustus*, *E. glaucus*, *E. sibiricus*, *E. villosus*, *E. virginicus*, *E. virginicus* var. *australis*; *Festuca idahoensis* Elmer; *Holcus lanatus*; *Hordeum gussoneanum*, *H. jubatum*, *H. jubatum* var. *caespitosum*, *H. brevisubulatum*, *H. nodosum*; *Lolium perenne*; *Phleum pratense*; *Poa pratensis*; *Sitanion hansenii*.

On Feb. 24, 1939, the first sori were noticed, although it is probable that by careful searching they might have been found before that date. On March 25 data were taken, recording the total number of plants and the number showing smut for each inoculation.

Of the 42 species and varieties inoculated, 11 showed stripe smut in varying percentages. These are listed in table 6, which also contains infection percentages indicating the relative efficacy of spores and sporidia as inoculum.

TABLE 6.—*Reaction of species of Agropyron, Elymus, Hordeum, and Sitanion when inoculated with spores and sporidia of Ustilago striiformis in the greenhouse*

Species	Acc. No.	Percentage of infection*		
		Check	Col. L-A (spores)	Col. L-B (spores)
<i>Agropyron caninum</i>	139	0	34	91
" <i>cristatum</i>	195	0	0	5
" "	210	0	0	0
" <i>inerme</i>	268	0	30	27
" <i>smithii</i>	308	0	11	0
" <i>subsecundum</i>	309	0	0	2
" "	138	0	29	5
" "	12	0	44	10
" "	13	0	11	34
<i>Elymus canadensis</i>	341	0	33	17
" " var. <i>robustus</i>	126	0	0	28
" <i>glaucus</i>	127	0	34	0
" "	342	0	29	6
" <i>sibiricus</i>	335	0	0	4
" "	338	0	33	44
<i>Hordeum nodosum</i>	169	0	7	0
" "	189	0	0	2
<i>Sitanion hansenii</i>	392	0	50	0
Average		0	19	15
				19

\* Based on plant counts.

The results of this inoculation experiment indicate that, in general, sporidia of opposite sex are just as effective for inoculum in greenhouse

studies of this race of *Ustilago striaeformis* as are spores. Considering the ease with which *U. striaeformis* may be cultured and the rapid growth of the cultures it seems that sporidia might well be used entirely for inoculation experiments, and the investigator would not have to be concerned with the necessity of maintaining a supply of viable chlamydospores.

On the basis of the results of this experiment it cannot be determined definitely whether collection L-A, from *Agropyron pauciflorum*, is pathogenically different from collection L-B, from *Elymus glaucus*.

A comparison of the data in table 5 with those in table 6 indicates to some extent that infection percentages obtained in the field were generally higher than those obtained in the greenhouse, even with the same grasses. Further experiments are necessary definitely to prove or disprove this. The smut sori begin to appear in inoculated grasses in the greenhouse as soon as or sooner than in the field, and data on a series of inoculations can be taken within 2 months after planting. It is thought that eventually inoculations of grasses with *Ustilago striaeformis* can be as reliably conducted in the greenhouse as can those with the various rusts.

#### Varietal Reaction of Selections and Collections of Slender Wheatgrass to *Ustilago striaeformis*

Considering the effect of stripe smut on its hosts, it is the opinion of the writer that this disease has the possibilities of becoming one of the most serious of slender wheatgrass and certain other grasses in the Northwest. As has been true in the research programs concerning other plant diseases, the search for resistant or immune strains of the host plants should be included in practical studies of the stripe smut of grasses.

Since the writer had several collections and selections of slender wheatgrass and since many others were available through the courtesy of cooperating agencies, it was decided to test these for relative susceptibility to *Ustilago striaeformis*. Thirty-four strains of slender wheatgrass were inoculated with collection L-B (from *Elymus glaucus*) of *U. striaeformis*. The seed was inoculated with an aqueous suspension of sporidia of opposite sex, and planted in nursery rows in the spring of 1938. Although the smut began to appear in a few weeks after seeding, the taking of data was deferred until about the middle of July, when it was noticed that many of the infected plants were dying due to inability to withstand drouth because of the shredded condition of the leaves, produced by the stripe-smut infection. The plants in each row were uprooted and data taken on a plant-count basis (Table 7).

As seen from table 7, there is surprisingly little resistance in the 34 selections and collections of slender wheatgrass inoculated with stripe smut. Only 6 selections appeared promising as resistant or immune stock: Acc. Nos. 74, 137, 249, 250, 252, 259. These results indicate that slender wheatgrass, as a species, is quite susceptible to stripe smut, and, such being the case, attention should be given in the grass improvement program to selections resistant to or immune from this disease.

TABLE 7.—Varietal reaction of selections and collections of slender wheatgrass to *Ustilago striiformis* from *Elymus glaucus*

Writer's Acc. No.	Other No.	No. plants	No. smutted	Percentage smut	
6		20	11	55.0	
56	Sel. from Wn. 279 <sup>b</sup>	84	58	69.0	
57		111	45	40.5	
58	Wn. 251	38	18	47.3	
59	Wn. 241	73	41	58.0	
60	Wn. 239	38	12	31.6	
61	Sel. from Wn. 279	60	24	40.0	
62	Wn. 249	296	90	30.4	
63	Wn. 242	76	23	30.2	
64	Sel. from Wn. 279	211	33	15.6	
65	do	114	41	35.9	
66	do	32	17	53.1	
67	do	85	77	90.5	
68	do	172	59	34.3	
69	Wn. 253	168	86	51.1	
70	Wn. 512	87	31	35.6	
71		156	52	33.3	
72	Wn. 435	57	36	63.1	
73	Wn. 434	99	65	65.6	
74	Wn. 472	187	14	7.4	
75	Sel. from Wn. 279	103	46	44.6	
83		106	43	40.5	
84		100	35	35.0	
137		a	0	0.0	
249	Var. "Grazier"	W. 3123 <sup>c</sup>	104	2	1.9
250	Var. "Fyra"	W. 3218		0	0.0
251		W. 2712	205	56	26.3
252		W. 934	13	1	7.7
253		W. 591	70	29	41.4
254	Var. "Mecca"	W. 3124	217	72	34.1
259		W. 879		0	0.0
288			138	53	38.4
306			150	44	29.3
324			59	29	49.1

<sup>a</sup> Number of plants was not determined in rows showing no smut.

<sup>b</sup> Numbers preceded by Wn. are of the Wash. Agr. Exp. Stat.

<sup>c</sup> " " " " " " " Pullman Unit of the Soil Conservation Service, Section of Nurseries.

### The Possibility of Seed Carriage in the Life History of *Ustilago striiformis*

Davis (10) has shown that as far as stripe smut in timothy is concerned, infection occurs from after-ripened spores in the soil. He showed that infection seldom, if ever, results from seed-borne spores or mycelium. Of 208 plants resulting from seed taken from infected plants, only one showed smut and, as Davis (10) points out, it was in one of the uncontrolled plots. He thus showed that seeds borne on infected culms do not produce infected seed, either as a result of penetration of the mycelium of the host plant into the ovaries on that plant or as a result of the lodging of wind-borne spores between the palea or lemma and the developing seed within.

It seems desirable in this connection to record here a simple preliminary experiment very strongly indicating that at least this new race of *Ustilago striiformis* on *Agropyron* and *Elymus* in the Northwest is seed borne. As

already explained several plants of slender wheatgrass, infected with stripe smut (original collection L-A), were transplanted from the field to the greenhouse where they continued to produce smutted leaves and culms. On each plant were a few culms, on which at least some seed was produced. Some of these seeds were harvested so as to obtain a smut-free stand of a susceptible host on which stripe smut could be propagated, it being remembered that Davis (11) had shown that, at least as far as the smut on timothy is concerned, the organism is not seed-borne. These seeds were sown in the nursery in the spring of 1937.

The writer was surprised to find that *every plant in the nursery row in which the seed had been sown was infected with stripe smut*, and not one survived to produce any seed.

This experience and the fact that, as has been shown above, seed inoculations have been so successful, indicates that at least this race of *Ustilago striaeformis* is certainly seed-borne. This aspect of the life history should be investigated with regard to other races of *U. striaeformis*.

#### DISCUSSION

A comparison of the results here reported on life-history and inoculation studies of *Ustilago striaeformis* with those of earlier investigators makes it obvious that the collections L-A and L-B, from *Agropyron pauciflorum* and *Elymus glaucus*, respectively, constitute a race of this smut fungus that is very different from any heretofore investigated. This race differs from other races that have been studied in its pathogenicity, process of spore germination, physiological requirements for germination, and its ability to grow saprophytically. Furthermore, aside from a very few fusions between atypical non-abstricted lateral sporidia observed by Davis (9), studies of this race provide the first information we have had regarding the sexuality of *U. striaeformis*.

The fact that the collections L-A and L-B of *Ustilago striaeformis* could not infect redtop, Kentucky bluegrass, orchard grass, and timothy proves that those collections are pathogenically distinct from any of the races described on these hosts. Inasmuch as no inoculation experiments have been heretofore reported dealing with collections of *U. striaeformis* on *Agropyron* and *Elymus* spp., it seems probable that collections L-A and L-B represent a new race of this smut species, with a comparatively wide host range of grasses in the genera *Agropyron*, *Elymus*, *Hordeum*, *Sitanion*, and perhaps others.

The production and abstraction of typical sporidia, which characterizes this race, has not been heretofore described for *Ustilago striaeformis*. The development of 2 or 3 or more germ tubes or promycelia from the same spore is also new to *U. striaeformis*. According to earlier descriptions of germination in this species, a single elongate promycelium is extruded through a crack in the spore wall, which bears lateral branches or "primary sporidia" that are not detached.

The after-ripening period of several months in a moisture-saturated atmosphere at room temperature, which Davis (9) has shown to be prerequisite to successful spore germination in the races of *Ustilago striiformis* that he studied, apparently does not apply to this new race on *Agropyron* and *Elymus* spp. in the Northwest. In the studies here reported, successful germination of fresh spores was nearly always possible, or if fresh spores failed to germinate well, several weeks' dry storage at room temperature usually sufficed to induce good germination. However, the fact that in a few cases fresh spores of this new race of *U. striiformis* failed to show more than a trace of germination, and the fact that in one instance a collection of this smut from Kentucky bluegrass showed 85 per cent germination after 2½ months' dry storage and without any after-ripening period under moist conditions, indicates that we do not yet have a complete understanding of the physiological requirements for germination of the spores of this smut fungus.

This new race of *Ustilago striiformis* is easily cultured and maintained on artificial media, which is in marked contrast to all other races studied heretofore in which the investigators could not induce the slightest saprophytic development. In the present studies vigorous, rapidly-growing cultures were easily obtained.

The data concerning the sexuality of the sporidia presented in this paper establish our first knowledge of the rôle of sex in the life history of *Ustilago striiformis*, wherein it is seen that this smut fungus, although, in the symptoms produced, differing widely from most smut fungi that have been so investigated, is, nevertheless, very similar to these with respect to sporidial fusions, development of infection hyphae, and the initiation of infection by these hyphae. In other words, it appears that the fundamentals of the life history of *U. striiformis* are not significantly different from those of other species of *Ustilago*.

Considering the extent to which the race of *Ustilago striiformis* on *Agropyron* and *Elymus* in the Northwest differs from those studied elsewhere and on other grasses, it might be considered that these races do not all belong to the same species; indeed, there might be some justification in treating this new race in the Northwest as a separate, probably new, species. However, the fact remains that the morphology of the spores of this race is not significantly different from that of the spores of races on other hosts. It is the opinion of the writer that, for this reason, and because they all produce the same symptoms on the host plants, the race of *U. striiformis* described in this paper should not be considered a valid species distinct from the type of *U. striiformis*.

Since the collections L-A and L-B, from *Agropyron pauciflorum* and *Elymus glaucus*, respectively, represent a new race of *Ustilago striiformis* it seems desirable to give it some designation. Davis (13) distinguished 4 physiologic races of *U. striiformis*: (1) forma *Phlei*, on *Phleum pratense*; (2) forma *Agrostidis*, on *Agrostis palustris*; (3) forma *Poae-pratensis*, on *Poa pratensis*; and (4) forma *Poae-annuae*, on *Poa annua*. Following Davis'

classification (12), and considering that this new race parasitizes several genera of the tribe Hordeae, it should perhaps be designated as *Ustilago striaeformis* forma *Hordei*.

Although experimental evidence is somewhat lacking, it seems probable that stripe smut is seed-borne. This supposition is supported by the fact that seed harvested from non-smutted spikes, borne on an infected plant of *Agropyron pauciflorum*, yielded 100 per cent smutted plants. Inasmuch as the spores are developed and liberated throughout the period of blossoming and seed development, it seems probable that during the development of the seed the stripe-smut spores become lodged beneath the palea and lemma and either remain dormant there until the seed germinates, or may germinate immediately and develop in the pericarp or hull of the seed a mycelium, which becomes dormant as the seed approaches maturity. Thus, this phase of the life history of *Ustilago striaeformis* probably is much the same as in some of the cereal smuts, such as *U. avenae* and *U. nigra*. Therefore, stripe smut in slender wheatgrass, and in other species of *Agropyron* and *Elymus*, as caused by the race *Hordei* of *U. striaeformis*, should be subject to control by the usual fungicides recommended for control of certain seed-borne smut fungi of cereals. Experiments are in progress to determine seed treatment methods of control of stripe smut under Northwest conditions.

#### SUMMARY

This paper deals chiefly with certain aspects of the life history, physiology, and pathogenicity of a new race of *Ustilago striaeformis* occurring on grasses of the genera *Agropyron* and *Elymus* in the Pacific Northwest.

No after-ripening period under moist conditions was found prerequisite to successful germination of the spores, such as has been previously found necessary (10, 11) in certain races of *Ustilago striaeformis*. The period of germinability extended over a period of at least 7 months.

The process of spore germination is described and illustrated. From the germinating spore 2 or 3 thick germ tubes emerge, rapidly elongate, and develop cross walls and branches. Typical elliptical sporidia are budded from this complex promycelium in abundance. These sporidia possess the ability to develop rapidly into large colonies on agar media. Any saprophytic existence has been heretofore unknown in *Ustilago striaeformis*.

The sporidia are unisexual, representing one or the other of two sex groups. When sporidia of opposite sex are mixed together, especially on non-nutritive agar, they fuse within a few hours. From each fused pair there arises a long, vigorous, aerial infection hypha.

The sporidia of this race of *Ustilago striaeformis* fuse readily with certain other species of *Ustilago*. Mating experiments with 12 pedigree monosporidial cultures of *U. bullata* and 4 of *U. striaeformis* showed: (1) The sporidia of *U. striaeformis* are highly compatible with those of *U. bullata*; (2) certain combinations are far more productive of infection hyphae than are others; and (3) in every case this greater number of infection hyphae involved the same sex group of *U. striaeformis*.

*Ustilago striaeformis* has been artificially cultured for the first time. Four monosporidial cultures (2 of each sex group) have been easily maintained on a variety of agar media. These cultures have continued a vigorous saprophytic development for over a year, having been transferred several times during this period. Although excellent growth is easily obtained on a variety of agar media, the optimum development has resulted on a 2 per cent agar containing 8 per cent dextrose, 4 per cent malt extract and 1 per cent peptone.

Inoculation experiments have been easily conducted, using aqueous suspensions of either spores or of sporidia of opposite sex as inoculum, by the partial vacuum method. High percentages of infection resulted on several species of *Agropyron*, *Elymus*, *Hordeum* and *Sitanion*. *Agropyron smithii*, *A. subsecundum*, *Elymus canadensis* var. *robustus*, *E. sibiricus*, *Hordeum jubatum*, and *H. nodosum* are reported as new hosts to *Ustilago striaeformis*, on the basis of inoculation experiments.

Inoculation experiments were completed in the greenhouse almost as successfully as in the field. In either case smut began to appear within 6 weeks after seeding.

Apparently, this race of *Ustilago striaeformis* is seed-borne, which has not previously been demonstrated for this species. Seeds taken from infected plants of slender wheatgrass produced only smutted plants.

Thirty-four selections and collections of slender wheatgrass were tested for resistance to this race of *Ustilago striaeformis*, with the result that most proved to be quite susceptible. Only 6 selections or collections appeared promising as resistant or immune stock.

The need for some designation of this new race is recognized, and it is recommended that it be known as race *Hordei*, or *Ustilago striaeformis* forma *Hordei*, in keeping with the classification of physiologic races of this species that Davis (12) has already initiated. In spite of the striking differences from other races of *U. striaeformis*, with regard to (1) spore germination and factors affecting the process, (2) pathogenicity, (3) culturability, (4) sexuality, and (5) seed carriage of the organism, this new race is indistinguishable, on the basis of spore morphology, from other races, and probably should not be considered a distinct species.

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# CORTICIUM AREOLATUM, THE CAUSE OF THE AREOLATE LEAF SPOT OF CITRUS

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Areolate leaf spot of *Citrus* is common in Surinam. Already, 20 years ago, the sour-orange stock of the nurseries was badly affected by this disease during long and heavy rainy seasons.

In 1929 Bondar (2) in Bahia described this leaf spot for the first time as "mancha areolada."<sup>1</sup> In his opinion it is the same leaf spot that occurs in Italy, and, according to Penzig, is caused by *Leptosphaeria citricola*.

Bitancourt and Jenkins (1) studied this disease again in 1935 and supposed the cause might be *Leptosphaeria bondari*. Infection experiments, however, were unsuccessful.

This *Leptosphaeria*, in Surinam, is very common on Citrus leaf spots. The same or a similar *Leptosphaeria* is found here as a common saprophyte on nearly every Cercospora leaf spot of the banana. It can be shown that the Citrus *Leptosphaeria* also is a saprophytic fungus.

The mycelium of the *Leptosphaeria* leaf-spot fungus grows rapidly and is, therefore, most easily isolated from young areolate leaf spots. This mycelium is entirely different from that of *L. bondari*. It resembles strikingly that of some primitive Basidiomycetes. Clamp connections are lacking.

To collect further and specific information about this parasite, I studied the epidermis of many of the very youngest leaf spots not yet necrotic. The germ tube was found to pierce the cuticle. Only once the spore was still attached to the germ tube (Fig. 4, H). It was a unicellular, thin-walled spore of basidiomycetous type.

It, therefore, seemed very probable, that areolate leaf spot of *Citrus* is caused by a representative of the Basidiomycetes.

## OCCURRENCE AND SYMPTOMS

Not all varieties of *Citrus* show the same susceptibility to attack by the areolate-leaf-spot fungus. Sour orange used for stock, suffers most severely. Grapefruit, pomelo, mandarin, and king also are susceptible. The common orange tree is fairly resistent, though, under heavy shade of *Erythrina glauca*, even with oranges, many leaves may be spotted during a long rainy season.<sup>2</sup> On lemon, lime, sucade, and kumquat, I never observed areolate leaf spot.

Only the very young, immature leaves are subject to infection during rainy weather. In Surinam, new shoots and twigs may appear on citrus

<sup>1</sup> In his original publication (p. 74) Bondar corrected "areolada" with ink into "aureolada." The term "areolate leafspot" thus originated from a misprint, but is used now exclusively. It seems to me better to perpetuate this and not to confuse the literature with the rehabilitation of the original name.

<sup>2</sup> In Surinam, full-grown orange trees, if not irrigated, suffer more or less from die-back. Under light shade of *Erythrina glauca*, the shade tree used here for coffee and caacno, dieback of Citrus may entirely be prevented, even without irrigation.

trees throughout the year, excepting the dry season. If the new twigs grow out during a fortnight with none or only a moderate rainfall, all the leaves remain permanently healthy. During continuously rainy weather, however, the new leaves become successively more and more spotted, and, ultimately, so much so that all are shed. By that time a grapefruit tree shows plenty of old, darkgreen, healthy leaves, only the new ones being spotted or shed. The full-grown trees of the susceptible varieties, therefore, suffer severely only in years of abnormally long, rainy seasons. In the nurseries of sour-orange stock, however, this disease causes much more trouble; in wet years, the plants may be badly defoliated if no control measures are taken.

The primary dead spots of  $\frac{1}{2}$ -1 mm. diameter appear, just as the leaf becomes full-grown, but still shows the light-green color of the immature leaf. In this stage the spots may begin to enlarge and continue to do so for many weeks during rainy weather.

The very first symptoms are small light-green spots on the young, not yet full-grown and still soft leaves. These spots die after some days and form the above-mentioned necrotic primary spots (Fig. 1, A). In heavily infected nurseries I counted 100 and more of these primary spots on a single leaf. If this stage is reached during days of heavy rain, most of the spots enlarge and the leaf is shed after some weeks. If, however, the weather is dry, all the spots remain permanently at this stage, causing no trouble of any importance.

The enlarging spots add every day one new ring to the spot. During 2 weeks I followed daily the growth of 12 spots. Every morning one new water-soaked ring about 1 mm. broad was found around the spot. During the day this ring discolors into brown and the tissue collapses. At the same time the leaf cells contiguous to the spot are impregnated by a yellowish gum. This gummed barrier is not yet tight in the evening of the first day, so that during the night, if sufficient moisture is present, the barrier is passed by the fungus and a new water-soaked ring is added. The rings of gummed cells do not collapse and form the concentric ridges so typical for areolate leaf spot. These ridges are more prominent on the lower than on the upper side of the leaf.

During dry weather the fungus stops growth and the gummed barrier is closed. Further spreading of the fungus inside the leaf is impossible. Sometimes, however, if wet weather prevails, the fungus may still find one or a few breaches to pass through. In this case it forms the curiously branched spots, that may be found sometimes. Usually, the spots show 10-20 rings, but in an extreme case I counted as many as 47 rings on a branched spot.

The enlarging of the primary spots seems to be more difficult than that of the ring spots. On most of the older spotted leaves several of these inconspicuous primary spots may be found. But even if they expand, they usually do so asymmetrically. Purely concentric spots are rare.

The color of the spots is light-brown; that of the ridges, dark-brown.

The upper surface is glossy, the lower dull. Around the spots the leaf is discolored, becoming yellowish.

During wet weather the lower side of many of the spotted leaves is covered with a whitish mildew (Fig. 1, B). There may be only a few patches outside the dead spots, sometimes, however, a big part, exceptionally the whole leaf, may be covered with it. Even the dead spots may show the mildew. These patches are nearly always very inconspicuous, especially on the light-

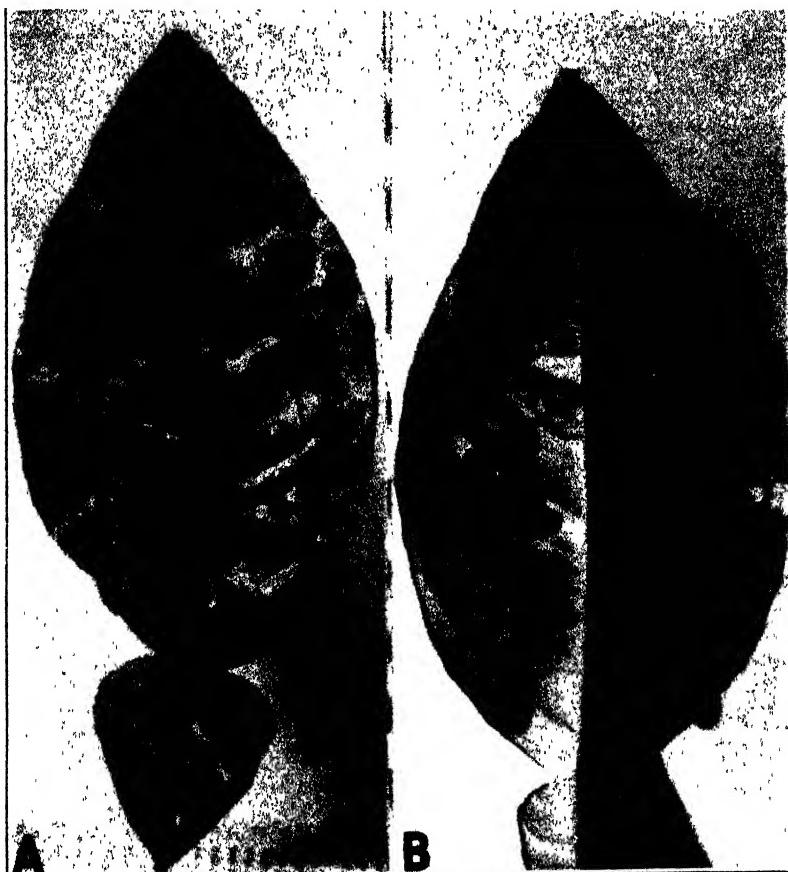


FIG. 1. A. Sour orange leaf with areolate leaf spots and 22 primary, not developed spots. B. Lower surface of sour orange leaf. On the right in the middle note the epiphylllic basidiophorous mycelium.

green, dull, lower leaf surface. It was Bitancourt who, during my visit to São Paulo, in October, 1938, called my attention for the first time to this very important symptom of areolate leaf spot. During very wet weather it sometimes happens, that even the upper surface of the leaf may show big patches of mildew, easily visible on this dark-green side of the leaf.

During wet weather the mycelium in the dead spots of the fallen leaves grows profusely out into the soil, and it is not without some effort that such a leaf can be detached from the underlying earth. Inspecting the lower

side of such a leaf, the spots appear to be covered with sand, and strands of hyphae covered with particles of sand hang down from the spots. As with other *Corticium*s, the areolate leaf-spot fungus is apparently a soil fungus. It is very probable, indeed, that it survives the long dry season in the soil in the form of sclerotia to reinfect the Citrus leaves at the commencement of the rainy season.

On young, green twigs primary spots may be found, as mentioned for the first time by Bondar (2). They never enlarge.

On the fruits I never detected even primary spots, though it is not impossible, that they may be found there.

The oldest parts of the spots are generally covered by small black points, the perithecia of *Leptosphaeria bondari*, a common saprophyte.

#### THE CAUSAL FUNGUS

The fungus is easily isolated. A piece of a young areolate leaf spot, about  $1 \times \frac{1}{2}$  cm., is transferred to slant agar (Sabouraud). Within an hour the fungus grows all around into the agar. After 15 hours a strong mycelium, 1 cm. wide, surrounds the piece. From the edges the pure mycelium may be transferred to new agar tubes. If a young, clean leaf spot be selected, no superficial disinfection is needed. I used only a disinfected knife and a sterile Petri dish to cut the piece.

After about 4 days the mycelium covers the surface of the slant agar in the test tube. It is first hyalin but discolors quickly into a greyish brown. A low, rough, aerial mycelium covers somewhat irregularly the surface of the agar. The mycelium grows over the free surface of the glass, where it forms a fine, transparent, closely adhering mycelium of one layer of hyphae. On this mycelium some strains produce plenty of sclerotia of about 1 mm. diameter; commonly, however, no sclerotia are formed in agar tubes. They are found most abundantly, when cultivated on sterilised potato slices (Fig. 2); but, even here, some cultures show only a few or no sclerotia at all.

Young sclerotia are whitish or light brown; later, they discolor into dark brown.

The sclerotia are formed by a clump of oidium-like, much branched, and curled hyphae, being lateral branches of the straight hyphae that creep over the glass. Its structure is pseudoparenchymatic. Not the slightest trace of a cortex is present. Even in the centre, the different oidium-like hyphae are easily distinguished, though the intercellulars are not wide. In the outer parts of the sclerotium some of the common hyphae may be found from which the swollen sclerotial hyphae arise.

I never found these sclerotia outside the pure cultures, but I strongly suspect that they are formed in the soil by the mycelium that grows out so abundantly from the spots of the fallen leaves.

On the potato slices a white, feathery, aerial mycelium appears consisting of strangely branched hyphae.

In the leaf spots the fungus fills up practically all the spaces between



FIG. 2. Sclerotia on the inner surface of a wide mouth Erlenmeyer flask. Culture on potato.

the mesophyll cells. Short side branches penetrate between the palisade cells. The hyphae here grow tortuously, forming knots.

During rainy weather this mycelium grows out from the spots and spreads over the surface of the living parts of the leaf, as mentioned above. It is the same cobweb-like mycelium, that grows over the glass surface in pure cultures. Here however, the oidium-like side branches do not clump to form sclerotia but spread on the surface, producing on the lateral branches many isolated, short basidia (Figs. 3 and 4 D). During continuous heavy rainy weather the basidia produce 4 sterigmata, 10–13  $\mu$  long, with a 3–3½  $\mu$

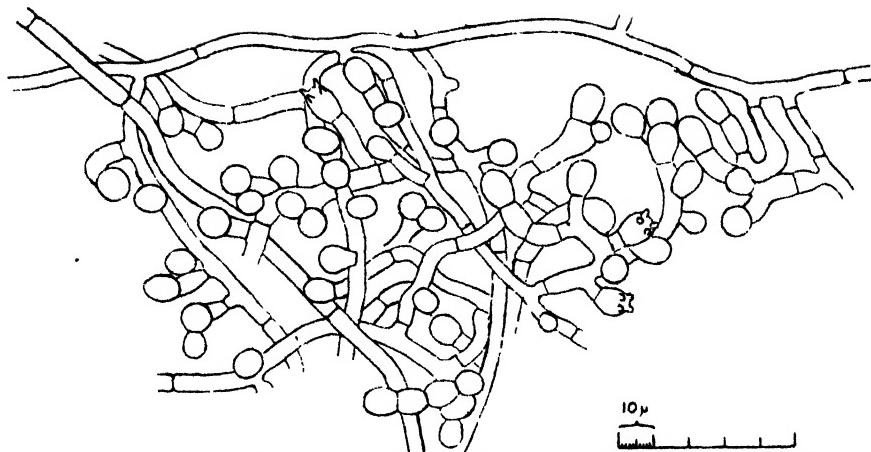


FIG. 3. Typical basidiophorous mycelium from the lower surface of the leaf.

broad base (Fig. 4, B and C). The spores are hyalin, smooth,  $5 \times 8-9 \mu$ , with a papilla (Fig. 4, E and F). There are regularly 4 sterigmata, only

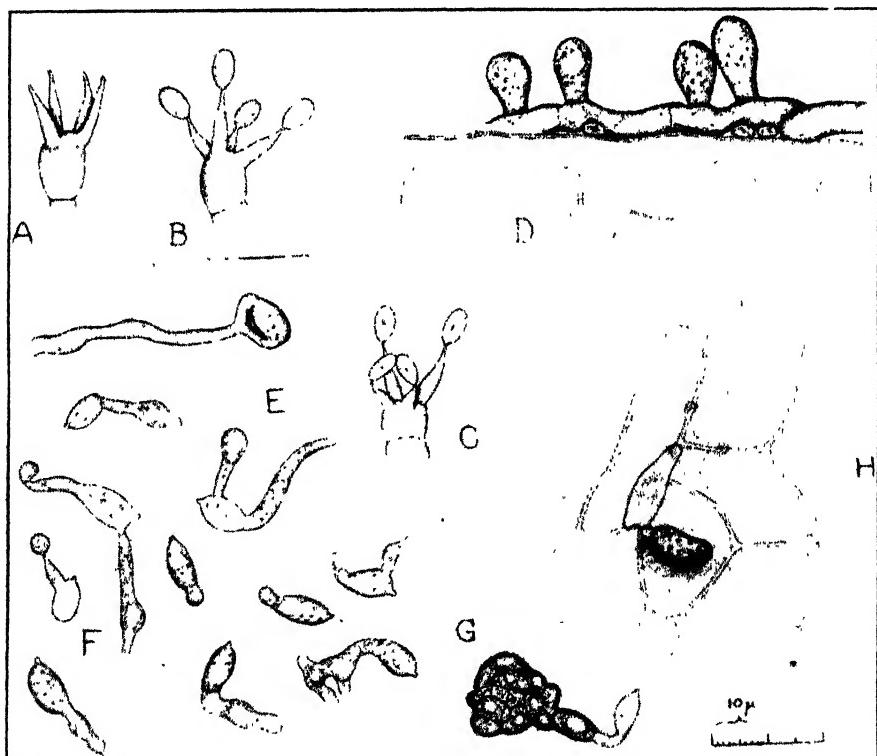


FIG. 4. A-C. Sporulating basidia. D. Epiphylllic mycelium with basidia. E. Ten basidiospores germinated on Sabouraud's agar. F. Basidiospores germinated in water. G. Basidiospore germinated on Sabouraud's agar forming a sclerotium, fourth day after germination. H. Germinated basidiospore on sour orange leaf showing a small appressorium, a flat subcuticular hypha, and hyphae in the palisade layer.

once I observed a basidium with 2. The basidia sporulate in groups, wherein every fifth to tenth may show sterigmata.

Bondar found this mildew commonly associated with areolate leaf-spot, and named it *Oidium citri*. Bitancourt confirmed this and supposed already in 1933 (in a letter to H. S. Fawcett), that this *Oidium* may be a *Corticium*



FIG. 5. A. Small flowerpot filled with moist sand. The buried sclerotia are grown out to form basidiophorous mycelia on the inner surface of the pot as well as on the sand. B. Moist flowerpot with basidiophorous mycelia grown out from about 12 areolate leaf-spots on the top of the inverted pot.

belonging as chief fructification to the leaf-spot fungus. The fact, however, that he couldn't find sporulating basidia nor clamp connections was the reason that he didn't mention this supposition in his publication written together with A. E. Jenkins in 1935 (1).

I collected the spores in Petri dishes on microslides. Inside the cover several pieces of leaves, covered with mildew, were fixed by droplets of water. If too many pieces are present in the same dish, the air becomes saturated with water and sporulation stops. In this case the epiphylllic hyphae grow out into a cotton-like aerial mycelium. Under adequate conditions the production of spores is most prolific, if the leaves are collected during continuous rainy weather. Then, within 24 hours, the microslides become covered with a whitish spore powder, easily visible to the naked eye.

Before I discovered the basidia on the leaf surface, I cultivated them from sclerotia produced on thick slices of potato in Petri dishes. The sclerotia on the inside of the cover were scraped together with a scalpel, brought into small flowerpots half filled with clean sterile sand, and buried 1-2 cm. deep. The whole was left moderately wet and uncovered, so that fresh air could circulate freely.

After 10-14 days the inside surface of the flowerpot showed big patches of a white mildew exactly like that on the leaves (Fig. 5, A). Sporulation, however, is rather more prolific on the flowerpots than on the leaves, apparently because the moisture is here more constant and may be regulated more conveniently. The basidia also are produced on the surface of the sand.

In the same manner the mycelium in the soil of a nursery or an orchard may grow out at the commencement of the rainy season and produce basidia on the surface. These basidiospores reinfect the nursery plants. A heavy outbreak of the disease, however, is not possible before plenty of leaves are covered with mildew produced by the dead spots.

It is not necessary to bury the sclerotia in the sand. The same thing happens if the sclerotia are put on a continuously and moderately moist inverted empty flowerpot. Instead of sclerotia I used with the same success young areolate spots cut out from spotted leaves (Fig. 5, B). The sclerotia and leaf spots, however, have to be covered during the first week with a small glass plate to retain the moisture.

These experiments show the following conditions necessary to the production of the basidiophorous mycelium: *a.* A well-nourished mycelium. *b.* A moderately but constantly moist substratum. *c.* An unhindered ventilation in the open air.

Here the temperature is not considered. For Surinam, with about the same temperature throughout the year, this factor is of no importance. The fact, however, that, according to Bitancourt, the sour orange in Santos suffers from this disease, whereas in the cooler climate of nearby São Paulo no areolate spot at all is known, shows that the minimum temperature may be fairly high. The areolate leaf-spot fungus needs a wet, tropical climate.

## INFECTION EXPERIMENTS

In a citrus nursery, even if badly infected with areolate leaf spot, all the young and tender leaves are free from spots. But when the leaf has just reached its normal size, the primary spots appear. On old leaves young growing spots never are found. It is clear, therefore, that only the young, tender leaves are subject to infection by basidiospores.

For the infection experiments, I used mycelia, sclerotia, and basidiospores. With all infection was successful.

To inoculate sclerotia and mycelia, I made 2-3 cm. cuts in old dark-green leaves, using a razor to make the cuts. Immediately small quantities of sclerotia or mycelia, cleaned carefully from adhering agar, were brought into the cuts. Two days later the edges appeared to be necrotic and later, every day, a new ring was added, producing the typical areolate leaf spots (Fig. 6). Inoculations with mycelium grown on nutrient agar from basidiospores had the same result.

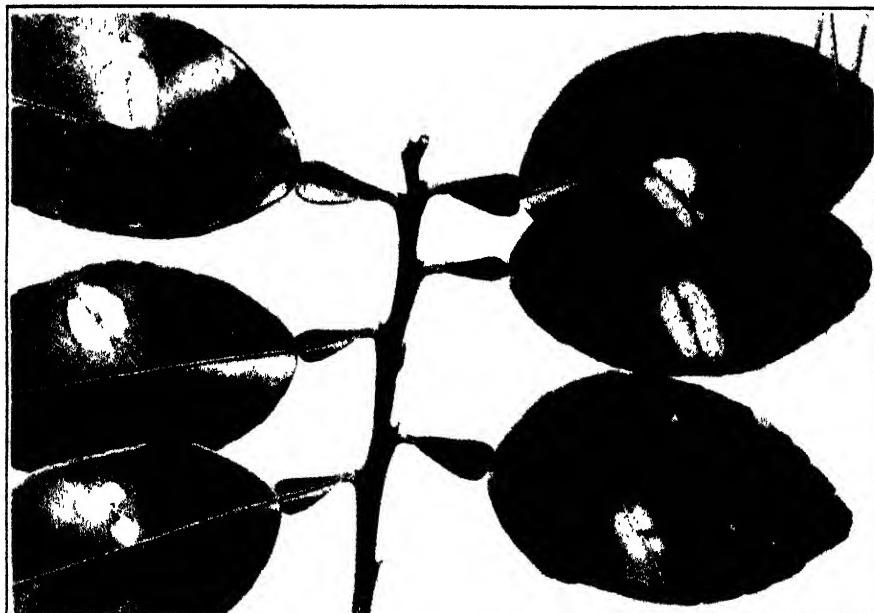


FIG. 6. Infection of old leaves with sclerotia brought into cuts freshly made with a razor.

To infect the young leaf with basidiospores I suspended the small flowerpots and spotted leaves covered with sporulating basidia above a developing new shoot of a young grapefruit plant. The whole was covered with a big flowerpot kept moist constantly. With the spores of both origins infection was successful. The young grapefruit plants were growing close to my laboratory and were free from leafspots for at least 10 months. Infection was also successful when the spores, shed from the epiphylllic mycelium in Petri dishes, were collected with a wet pencil and brought on the leaves.

These inoculations, however, were commonly less effective, than those with spores directly shed on the leaves.

The experiments have shown, that only the youngest leaves of less than half the full-grown size take infection. Both sides of the leaf are susceptible. The incubation time is about 14 days.

In water and nutrient agar the basidiospores germinate easily within 12 hours. The germ tubes grow very slowly, forming—especially on nutrient agar—irregular swellings and windings, typical for the germ tubes of many parasitic fungi.

On nutrient agar after 4–5 days the germ tube forms a small sclerotium (Fig. 4, G). From this sclerotium the hyphae grow out and spread over the agar, producing the mycelium.

About the same happens if the fungus enters the leaf. The basidiospore germinates immediately, producing a minute appressorium that pierces the cuticle (Fig. 4, H). The resultant germ tube then grows as a flat hypha for a short distance between the cuticle and the epidermal cells and then

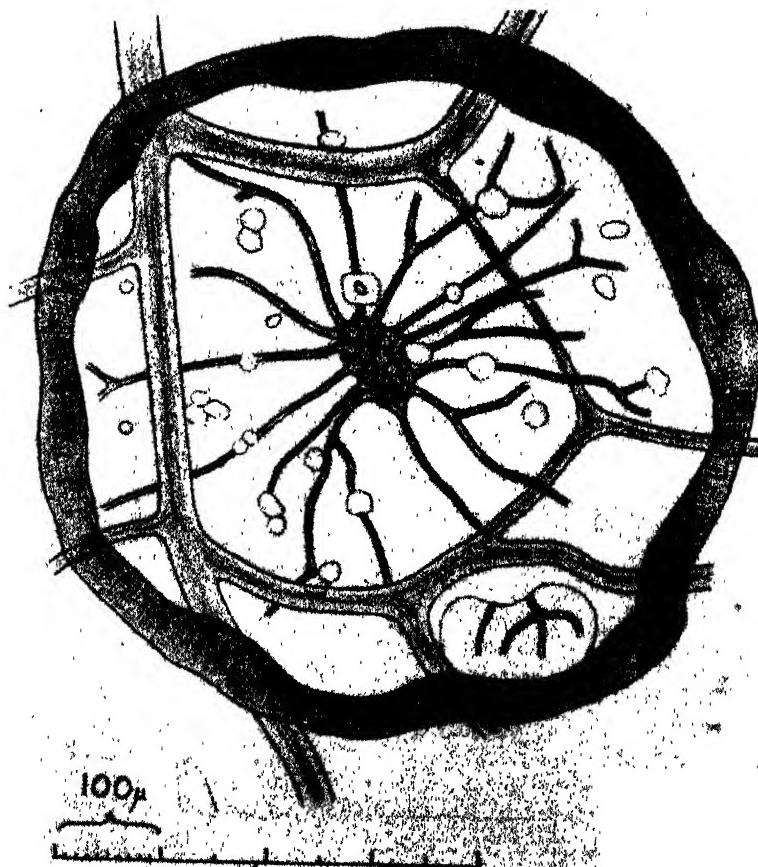


FIG. 7. Primary spot with the small initial sclerotium and the spreading hyphae. Gummed ringwall, nerves, and crystal cells.

enters the deeper leaf tissue by way of the intracellular space between epidermal cells.

In the mesophyll just below the point of spore germination, a sclerotium is formed that kills the tissue when the leaf is just full-grown. Then all round the edges of the sclerotium the hyphae spread radially into the dead tissue, and, if the weather is wet enough, this primary spot grows out to an areolate leaf spot as described above (Fig. 7).

The fungus doesn't form a true hymenium. The basidia are scattered over the side branches of the fertile mycelium (Fig. 3) that covers the substratum like a cobweb.

Accepting the reclassification of the *Thelephoraceae* by Burt (1914) the fungus is a *Corticium* and not a *Hypochnus* (with echinulate spores). I, therefore, propose to name the fungus causing areolate leaf-spot of Citrus ***Corticium areolatum*, nov. spec.**

#### Description of the Fungus

Vegetative hyphae brown, 5-8  $\mu$ , maximal 10  $\mu$  thick, distance of septa 50-200  $\mu$ , exceptionally 320  $\mu$ , clamp connections lacking. Hyphae in the leaf spot tortuous and knotty, epiphylllic hyphae straight, light brown, with hyaline ramified side branches of swollen cells bearing basidia. True hymenium and pileus lacking. Basidia short 10-14  $\times$  8-10  $\mu$ , sterigmata 4, exceptionally 2, 10-13  $\mu$  long, basis of the sterigmata 3-3½  $\mu$  thick, spores smooth, hyaline, papillate 5  $\times$  8-9  $\mu$ .

Sclerotia whitish or light brown, later dark brown; diameter 1 mm., generally somewhat flat, consisting of a clump of swollen hyphae, cortex lacking. Observed only in pure cultures on the surface of the glass container.

Wet weather parasite on citrus leaves, especially on sour oranges, but also on grapefruit, pomelo, king, mandarin. Fairly immune are oranges, immune lemon, lime, succade, kumquat.

In Surinam in the coastbelt, causing brown leaf spots, showing typical concentric rings, called "ringvlekkenziekte." Known as "mancha areolada" (mancha aureolada) and "areolate leaf spot," in Brazil from Bahia to Paraná and also in Venezuela (H. S. Fawcett, Citrus diseases, 1936).

Hyphis vegetis brunneis, 5-8 (-10)  $\mu$  crassis, in maeulis tortuosus nodosisque, epiphyllis rectis, pallide brunneis; ramis lateralibus hyalinis, ramosis, e cellulis inflatis basidiiferis compositis; hymenio et pileo deficientibus; basidiis curtis, 10-14  $\times$  8-10  $\mu$ ; sterigmatibus 4, rare 2, 10-13  $\mu$  longis, ad basim 3-3.5  $\mu$  crassis; sporis levibus, hyalinis, papillatis, 5  $\times$  8-9  $\mu$ ; sclerotis in culturis pallidis dein brunneis, 1 mm. diam., fere leniter deplanatis. Maeulas in foliis Citri producens, Surinam.

#### CONTROL

In nurseries of sour orange stock this very troublesome disease may effectively and economically be suppressed by collecting and burning all the spotted leaves. In Surinam this work is done by women and children.

If a nursery has to be sprayed for seab (*Elsinoë fawcetti*), areolate leaf-spot is controlled too. Bordeaux mixture kills and impedes the development of the sporulating epiphylllic mycelium.

During a long continuous rainy season grapefruit orchards suffer badly from this disease. A thorough spraying of the soil under the trees in the beginning of the rainy season may prevent or retard reinfection from the soil. If, in spite of this treatment, the new leaves are heavily spotted before the end of the rainy season, the spotted leaves have to be sprayed during dry days, especially on the lower side.

AGRICULTURAL EXPERIMENT STATION

PARAMARIBO, SURINAM

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PRELIMINARY SEROLOGICAL STUDIES OF PHYMATOTRICHUM OMNIVORUM<sup>1</sup>

R. W. CUMLEY AND G. W. GOLDSMITH

(Accepted for publication Sept. 18, 1939)

Although the cotton root-rot fungus has been the subject of numerous reports appearing during the past 30 years, the life history, including the perfect stage, has not been established. Both Shear (4) and Duggar (1) placed the fungus in the Hyphomycetes. Later, Shear (5) reported hyphal connection between diseased cotton and osage orange, upon which sporophores occurred, and assigned to the fungus the name *Hydnellum omnivorum*. This result has not been confirmed. Taubenhaus and Ezekiel (6) reported negative results. Recently, Presley and Thom (3) have made the suggestion that the fungus is to be regarded as a Gasteromycete, and the spore mats, previously considered conidial, are in reality the puffball sporophores. It is obvious that the correct classification of the fungus and interpretation of the structures would be of considerable theoretical and practical importance.

A preliminary serological study was undertaken to determine the relationship of *Phymatotrichum omnivorum* (Shear) Duggar to the various members of different groups of fungi. Two methods of securing material were followed. In one the young growing sporophores were collected in the field and immediately washed and dried over sulphuric acid at 50° C., and stored in sterile glass jars at 5° C. The following were prepared in this way: *Tyromyces palustris* (Berk. & Curt.) Murrill, *Psalliota silvatica* (Schaeff.) Quél, *Clitocybe illudens* Schw., *Lycoperdon gemmatum* Batsch, *Calvatia cyathiformis* (Bosc.) Morgen, *Secotium acuminatum* (Mont.), *Ustilago maydis* (DC.) Cda.

In the second method pure cultures of fungi were grown in liquid culture, removed when the growth was well developed over the surface, washed, and dried under the conditions just mentioned. By this method *Rhizopus nigricans* Ehrenberg, *Aspergillus niger* group Thom and Church, *Penicillium luteum* group Thom, *Hormodendron cladosporioides* (Fres.) Sacc., *Fusarium* sp., *elegans* section Gilman and Abbott, *Sclerotium rolfsii* Sacc., and *Phymatotrichum omnivorum* (Shear) Duggar were obtained.

## MATERIALS AND METHODS

*Culture Medium for Phymatotrichum omnivorum*.—The fungus mats

<sup>1</sup> Contribution from The Clayton Foundation.

were grown on an artificial nonprotein medium composed of the following materials dissolved in the order given:

Distilled water, 1000 cc.; dextrose, 40.00 g.; ammonium nitrate, 1.8 g.; di-potassium phosphate, 1.35 g.; magnesium sulphate, 0.75 g.; potassium chloride, 0.15 g.; and iron chloride (0.5 per cent solution), 0.25 cc.

Cultures were grown at room temperature in 2-liter Erlenmeyer flasks containing about 400 cc. of culture media. The fungus mats were collected at approximately 1 month after seeding.

*Collection of Fungus Mats.*—The mats were recovered by decanting the culture medium and pouring the mat into a large bowl of tap water. After 2 or 3 rinsings in tap water, the mat was rinsed twice in distilled water and dried rapidly in an incubator at 40–45° C. About 6 hours, and never more than 12, were required to get the material to constant weight. The mats were then placed in air-tight jars and stored at 5° C.

*Preparation of Injection Materials.*—Injection antigens were prepared in a manner similar to the technique of Link and Wilcox (2) working with species of *Fusarium*, *Sclerotinia*, and others. The technique employed was as follows: Physiological saline (.85 per cent NaCl) was added to the dried and powdered fungus material in the proportion of approximately 33 parts saline to 1 of fungus. The mixture was shaken thoroughly and placed in the ice-box at 5° C. for about 18 hours. During the course of the extraction the material was shaken several times. After extraction, the mixture was centrifuged and the clear amber-color supernatant liquid collected by decantation. To this extract was added merthiolate solution (1:1000) in the proportion of 9 parts of extract to 1 of merthiolate. The resulting fluid constituted the intravenous injection material and was preserved in the ice-box. This solution contained 0.0135 gm. fungus powder per cc.

The material for intraperitoneal injection was prepared from the residue remaining when the aforementioned extract was collected, after centrifugation. This residue was dried and weighed. It was then ground extremely fine in a mortar and resuspended in saline in the same proportions as in the preceding extraction, addition of merthiolate and preservation at 5° C. being the same as already described. Unlike the prior extraction, this mixture never assumed an amber color, but remained a dull gray caused by the suspended particles. This suspension contained 0.03 g. of preextracted fungus powder per cc.

*Inoculation of Rabbits.*—Three rabbits were inoculated 6 times, intravenously, with the above described solution and, intraperitoneally, with the suspension. The injections were given at about 4-day intervals. Table 1 is a presentation of the immunization schedule.

Seven days after the last injection the animals were starved for 24 hours and then bled from the heart. The amount of blood taken from the rabbits varied from 15 to 45 cc. The blood was allowed to remain in the ice-box overnight, after which the serum was pipetted into sterile containers and kept at 5° C., preserved with merthiolate solution. These sera were the anti-

TABLE 1.—*Immunization schedule followed in inoculating 3 rabbits 6 times intravenously and intraperitoneally with injection antigens*

Date	Rabbits						Remarks	
	Number 1		Number 2		Number 3			
	Intravenous (solution)	Intraperitoneal (suspension)	Intravenous (solution)	Intraperitoneal (suspension)	Intravenous (solution)	Intraperitoneal (suspension)		
3-12-38	cc.	cc.	cc.	cc.	cc.	cc.		
3-12-38	1.0	1.5	1.0	1.5	1.0	1.5		
3-15-38	2.0	2.5	2.0	2.5	2.0	2.5		
3-18-38	3.0	2.0	3.0	2.5	3.0	3.0		
3-21-38	4.0	4.5	4.0	3.0	4.0	4.5	No. 1 had large knot on belly and was sick after shots.	
3-25-38	4.0	3.0	4.0	4.0	4.0	2.5	Small knots on belly of each rabbit. Knots on No. 1 smaller.	
3-28-38	5.0	5.0	5.0	5.0	5.0	4.0	All animals bled from heart.	
4-4-38								

*Phyamatotrichum* sera that were employed in the subsequent tests with numerous fungus antigens.

*Preparation of Test Antigens.*—The antisera were tested with antigens prepared from 14 different genera of fungi, either collected in the field or grown on suitable synthetic media. Before extraction, the material was thoroughly dried, powdered, and weighed. The extracts used in the tests differed from the injection extracts in that the test antigens were prepared from dry powders, previously extracted with ether. The purpose of this extraction was to remove lipoids that have been thought to interfere with the specificity of the precipitin reaction.

The material prepared from each species was extracted 4 times with ether, after the technique of Link and Wilcox (2). This technique was as follows: "Ether was added to the desired amount of powder, shaken occasionally and allowed to act for 1-15 hours at 25° C. After centrifugation this ether was decanted and fresh added. Usually 3 or 4 changes sufficed to give a fat-free test when a drop of ether was evaporated on a watch crystal. The ether was then decanted and the powder thoroughly dried preliminary to saline extraction." Table 2 is a presentation of the various species employed here and an account of the percentage of ether-soluble material removed during the 4 ether extractions.

TABLE 2.—Data regarding ether-soluble constituents of several species of fungi

Name of fungus	Wt. of powder before ether-extraction	Wt. of powder after ether-extraction	Percentage ether-soluble constituents removed by extraction		
			Grams	Grams	Percent
<i>Aspergillus niger</i>	3.98	3.88		2.40	
<i>Calvatia cyathiformis</i>	5.00	4.84		3.20	
<i>Citocybe illudens</i>	5.00	4.65		7.00	
<i>Fusarium elegans</i>	0.67	0.38		43.30	
<i>Hormodendron cladosporioides</i>	5.00	4.62		7.50	
<i>Lycoperdon gemmatum</i>	5.00	4.82		3.50	
<i>Penicillium luteum</i>	5.00	4.27		14.50	
<i>Phyamatotrichum omnivorum</i>	5.00	4.60		8.00	
<i>Psalloota sylvatica</i>	5.00	4.20		16.00	
<i>Rhizopus nigricans</i>	2.00	1.75		12.50	
<i>Sclerotium rolfsii</i>	0.23	0.21		8.60	
<i>Secotium acuminatum</i>	5.00	4.70		6.00	
<i>Tyromyces palustris</i>	5.00	4.02		19.50	
<i>Ustilago maydis</i>	5.00	4.79		4.10	

After the ether had been thoroughly removed from the fungus powder, 0.85 per cent saline was added in the proportion of 1 g. of powder to 40 cc. of saline. The mixtures were shaken at frequent intervals and remained in the refrigerator at 5° C. for 3 days. They were then centrifuged and the clear supernatant solution collected and filtered. The residue was quickly dried and weighed. Hence, the amount of material that had gone into solution during the course of extraction could be calculated. Table 3 presents the data regarding this saline-extraction of the pre-ether-extracted

TABLE 3.—Data regarding solubility in saline of fungal powders previously extracted with ether

Name of fungus	Powder pre-extracted with ether	Saline added	Extract removed	Residue remaining	Material in extract	Dilution of material in extract
	Gram	cc.	cc.	Gram	Gram	Gram/cc.
<i>Aspergillus niger</i>	1.000	40.0	33.0	0.815	0.185	1: 178.5
<i>Catara crathiformis</i>	1.000	40.0	32.0	0.670	0.330	1: 97.0
<i>Chitogae illudens</i>	1.000	40.0	36.0	0.327	0.673	1: 53.5
<i>Fusarium elegans</i>	0.375	14.4	10.0	0.272	0.103	1: 97.2
<i>Hormodendron cladosporioides</i>	1.000	40.0	35.0	0.835	0.165	1: 212.1
<i>Lycoperdon gemmatum</i>	1.000	40.0	33.0	0.592	0.408	1: 80.8
<i>Penicillium tareum</i>	1.000	40.0	33.0	0.576	0.424	1: 77.9
<i>Phycomyces omnivorum</i>	1.000	40.0	36.0	0.660	0.340	1: 105.8
<i>Psallotra stratica</i>	1.000	40.0	35.0	0.382	0.618	1: 56.6
<i>Rhizopus nigricans</i>	1.000	40.0	32.0	0.740	0.260	1: 123.1
<i>Sclerotinia rolfsii</i>	0.210	8.4	7.2	0.157	0.053	1: 136.0
<i>Secotium acuminatum</i>	1.000	40.0	34.0	0.662	0.338	1: 100.5
<i>Tyromyces palustris</i>	1.000	40.0	31.0	0.440	0.560	1: 55.4
<i>Ustilago maydis</i>	1.000	40.0	34.0	0.832	0.168	1: 202.2

TABLE 4.—*Precipitation reactions of various antigens to anti-Phymatotrichum omnivorum serum*

Antigens	Antigen dilutions						
	1/1000	1/2000	1/4000	1/6000	1/8000	1/12000	1/16000
<i>Phymatotrichum omnivorum</i>	+++	++	-	-	-	++	-
<i>Lycoperdon gemmatum</i>	++	++	++	++	++	-	+
<i>Secotium acuminatum</i>	++	++	++	++	++	++	++
<i>Catathia cyathiformis</i>	++	++	++	++	++	++	++
<i>Penicillium luteum</i>	++	++	++	++	++	++	++
<i>Hormodendron cladosporioides</i>	++	++	++	++	++	++	++
<i>Tyromyces palustris</i>	+	+	+	+	+	+	+
<i>Sclerotium rolfsii</i>	++	++	++	++	++	++	++
<i>Peniophora sylvatica</i>	+	+	+	+	+	+	+
<i>Fusarium elegans</i>	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	-	-
<i>Rhizopus nigricans</i>	-	-	-	-	-	-	-
<i>Oltocybe illudens</i>	-	-	-	-	-	-	-
<i>Ustilago maydis</i>	-	-	-	-	-	-	-

powders. After having computed the quantity of powders in the various extracts, the dilutions of all were adjusted, by the approximate additions of saline, to the ratio of 1: 250. Merthiolate solution (1: 1000) was then added to the solutions as a preservative. These extracts constituted the test antigens employed in the subsequent complement fixation and precipitin tests.

*The Precipitin Test.*—The ring precipitin test of Ascoli was applied to the antisera, in the attempt to differentiate the antigens prepared from the several fungus species. In this test the antiserum was carefully placed in the bottom of 2" × ½" precipitin tubes, to a depth of about ¼". The antiserum was held at the same dilution throughout the 12 tubes in the rack. The antigens were diluted serially, beginning with 1: 250 (the stock solution) and proceeding up to 1: 128,000. The antigen of the proper dilution was layered on top of the antiserum, care having been taken not to allow the 2 reagents to mix at the interface. Approximately the same quantities of antigen solution and antiserum were used in each tube. Readings were taken at frequent intervals up to 3 hours. The 2-hour reading appeared to be the most consistent and is used in the results reported in table 4. The faintest perceptible ring is indicated in the results as a +, whereas a heavy, thick, opaque ring is indicated as a ++++. Negative results are indicated as —.

*The Complement Fixation Test.*—Before the complement fixation tests could be executed, the antigens were titrated for anticomplementary activity. This was accomplished by holding constant the amounts of complement (guinea pig serum), hemolytic antigen (2 per cent sheep cells), and hemolytic amoebocyte (anti-sheep cell serum from rabbit), and serially diluting the antigen to be tested. The results of this test are shown in table 5. Neg-

TABLE 5.—Titration for anticomplementary activity of antigens

Species antigen	Antigen dilution						
	1: 250	1: 500	1: 1000	1: 2000	1: 4000	1: 8000	1: 16,000
<i>Aspergillus niger</i>	—	—	—	—	—	—	—
<i>Calvatia cyathiformis</i>	—	—	—	—	—	—	—
<i>Clitocybe illudens</i>	—	—	—	—	—	—	—
<i>Fusarium elegans</i>	—	—	—	—	—	—	—
<i>Hormodendron cladosporioides</i>	—	—	—	—	—	—	—
<i>Lycoperdon gemmatum</i>	++	++	—	—	—	—	—
<i>Penicillium luteum</i>	—	—	—	—	—	—	—
<i>Phymatotrichum omnivorum</i>	—	—	—	—	—	—	—
<i>Psalliota silvatica</i>	+++	+++	+++	+++	++	+	—
<i>Rhizopus nigricans</i>	—	—	—	—	—	—	—
<i>Sclerotium rolfsii</i>	—	—	—	—	—	—	—
<i>Secotium acuminatum</i>	—	—	—	—	—	—	—
<i>Tyromyces palustris</i>	—	—	—	—	—	—	—
<i>Ustilago maydis</i>	++	—	—	—	—	—	—

TABLE 6.—Complement fixation reactions using anti-*Phymatotrichum omnivorum* serum and several fungal antigens

Fungal antigens	Antiserum dilutions							1/160
	1/6	1/9	1/12	1/18	1/24	1/36	1/48	
<i>Phymatotrichum omnivorum</i>	+++	+++	+ +	+++	++	+	+	-
<i>Lycoperdon gemmatum</i>	++	+	-	-	-	-	-	-
<i>Serotium acuminatum</i>	++	++	-	-	-	-	-	-
<i>Calvatia cyathiformis</i>	++	++	-	-	-	-	-	-
<i>Fusarium elegans</i>	++	++	-	-	-	-	-	-
<i>Ustilago maydis</i>	..	+++	++	-	-	-	-	-
<i>Clitocybe illudens</i>	..	++	+	+	+	-	-	-
<i>Sclerotium rolfsii</i>	++	++	-	-	-	-	-	-
<i>Hormodendron cladosporioides</i>	++	++	-	-	-	-	-	-
<i>Tyromyces palustris</i>	..	..	-	-	-	-	-	-
<i>Penicillium luteum</i>	..	..	-	-	-	-	-	-
<i>Rhizopus nigricans</i>	..	..	++	-	-	-	-	-
<i>Apergillus niger</i>	..	..	++	-	-	-	-	-

tive — results indicate no interference with the action of complement, whereas +++, indicates considerable and serious interference.

Because of the fact that the antigen prepared from *Psalliota silvatica* was anticomplementary in a dilution of 1:8000, it was discarded and not tested with the subsequent complement fixation reactions. In order to avoid any possible anticomplementary action indicated by the *Ustilago maydis* and *Lycoperdon gemmatum* reactions, and at the same time to keep all of the antigens of the same strength, the solutions were all diluted to the ratio of 1:1500. With these antigens, the antisera were tested by the complement fixation technique. The antigen was held at constant dilution and the anti-serum was serially diluted from 1:6 to 1:160. Preliminary tests with the homologous antigen indicated that the titre of complement fixation did not exceed that figure. In table 6, where the results of the several tests are compiled, +++, indicates complete complement fixation, and — indicates no fixation of complement.

#### RESULTS

*The Precipitin Test.*—The precipitin test was employed with all 3 of the rabbit sera. The serum of rabbit No. 1 was cloudy and was found unsuitable for the test. The serum of rabbit No. 3 gave a precipitate with saline. Several titrations were made to determine the saline dilution that would not form a ring with the antiserum, without success. Rabbit No. 2, however, yielded a serum that was neither cloudy nor affected by saline. Furthermore, this serum precipitated the homologous antigen in high dilutions. The results reported in table 4 are compiled from many precipitin tests executed on this antiserum. In this table, one may observe that antigens prepared from *Lycoperdon gemmatum*, *Secotium acuminatum*, and *Calvatia cyathiformis*, in the several tests conducted on the rabbit No. 2 antiserum, always reacted more nearly as did the *Phymatotrichum* antigen than did any of the other antigens tested. Hence, one may conclude tentatively that *Phymatotrichum omnivorum* is more closely related serologically to the puffballs than to any of the other forms represented in these tests.

*The Complement Fixation Test.*—In the complement fixation tests the sera of rabbits No. 2 and No. 3 yielded complement fixing antibodies in such low dilutions that they could not be used. The serum of rabbit No. 1 contained complement fixing antibodies in an antiserum dilution of 1:160, when tested against the homologous antigen. Consequently, this serum was employed in testing the relations of the various antigens to the antigen of the cotton-root-rot fungus. The results of these tests are shown in table 5. One may observe from this table that the puffball forms, viz., *Lycoperdon gemmatum*, *Secotium acuminatum*, and *Calvatia cyathiformis* again appear to be more nearly related to *Phymatotrichum omnivorum* than do any of the other genera tested.

By comparing the results in table 5 with those of table 6, one may readily observe that some of the species do not assume the same ranks in the 2 tests.

This is relatively inconsequential and is to be expected when use is made of 2 different sorts of tests. Indeed, 2 tests of the same sort will often present minor discrepancies. The most important and significant feature of these tests is that in both cases the 3 puffballs are ranked closer to the *Phymatotrichum omnivorum* than are the other genera. This evidence should serve to establish, at least presumptively, the serologic relation of the cotton-root-rot fungus.

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#### A WHITE ROOT ROT OF APPLE TREES CAUSED BY CORTICIUM GALACTINUM

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#### INTRODUCTION

Very frequently, in the investigations of apple root diseases, white fungi have been found associated with dead or dying roots. These fungi usually have proved to be saprophytes growing on dead or nearly dead roots. In 1932, however, a white fungus that appeared to be an active pathogen was observed growing on the roots of an apple tree at Heards, Virginia. Since that time studies on this fungus have established its active parasitism on apple-tree roots and its identity as *Corticium galactinum* (Fr.) Burt.

Von Schrenk<sup>1</sup> in 1902 published a brief note on a root rot, apparently identical with this disease, caused by *Thelephora galactina* Fr. (now a synonym of *Corticium galactinum* (Fr.) Burt). In 1909, Von Schrenk and Spaulding<sup>2</sup> stated that this fungus occurs commonly as a root parasite of oaks in various parts of the Ozark Mountains and that it spreads from oaks to fruit trees when the latter are planted on recently cleared land. No reference in the literature to this disease has been found since the publication of these two short notes.

<sup>1</sup> Von Schrenk, H. A root rot of apple trees caused by *Thelephora galactina* Fr. *Bot. Gaz.* 34: 65. 1902.

<sup>2</sup> Von Schrenk, H. and P. Spaulding. Diseases of deciduous forest trees. U. S. Dept. Agr., Bur. Plant Ind. Bull. 149. 1909.

## SYMPTOMS

Usually the disease starts at the collar or on larger roots and advances rapidly outward on smaller roots. Often the killing is so rapid as to kill the larger roots near the collar and girdle the tree while the distal portion of the roots may be still alive. The killing is usually so rapid and complete that the presence of the disease in the roots is manifest only in the top by the sudden death of the whole tree. On the other hand, the action of some slower developing root-disease fungi, such as *Xylaria*, is manifested in the top by weak limbs on the side of the tree above the diseased roots.

In the first stage of attack there is a growth of white hyphal strands on the surface of the root. As the fungus grows this surface layer becomes thicker and thicker till a dense weft of mycelium covers the surface of the root (Fig. 2, A and C). The fungus meanwhile gradually penetrates the epidermis, then the cortex and the cambium, and finally the wood, causing a white wood rot.

The cambium is not uniformly killed. This is shown by the zonate spots on the wood where the bark has been removed (Fig. 4, B). In some instances the margins of the spots are surrounded by incipient callus formation about the areas of killed cambium. In other cases where the host tissue surrounding the killed area is still more active, growth and enlargement take place, which result in a very peculiar and distinctive-looking root bearing pits and bumps over the surface (Fig. 4, A). Sometimes there is another manifestation of the disease resulting in a general hypertrophy of the root at the junction of the diseased and healthy areas.

## DISTRIBUTION

The disease was observed at Heards, in 1932, and since that time other trees in the same orchard have been killed. Affected trees have also been observed in the fruit regions near Middletown, Luray, and Leesburg, Va., Beltsville, Md., Greenville, Tenn., Bridgeville, Del., and Bedford, Ind. Von Schrenk<sup>3</sup> noted that root rots of apple trees were abundant in Kentucky, Missouri, Illinois, Arkansas, Oklahoma, and West Virginia, and considered *Thelophora galactina* one of the chief causes. The disease has been observed in a relatively small number of orchards but distributed over a wide area. Its presence has been confined to orchards that were set on newly cleared land or orchards in close proximity to a woods.

At various times since 1932, field surveys of root rots of apple trees have been made in many of the fruit regions in the eastern part of this country to study the types of root disturbances and their relative prevalence in the different regions. Very little survey work has been done, however, in the Ozark region where the disease was first reported. This present work has not been intensive enough in any one region to determine as fully as desired the prevalence, severity, and relation of the disease to environment. Reports from orchardists and oral reports from other investigators indicate

<sup>3</sup> See footnote 1.

that a thorough survey would show it to be more common and important than is realized at present.

#### PATHOGENICITY

Observations of diseased orchards and the gaps resulting from the death of trees indicate that the disease is more serious after they are 14 to 18 years old than in the case of younger trees. In one case a 3-year-old tree standing in the nursery was successfully inoculated in 1937. When inspected 1 year later no definite necrosis was found but the bark surface was abnormally rough and there was cortical thickening, as though cork tissue had effectively cut off and completely healed an extensive area nearly encircling the root where the fungus had initiated infection. On the other hand, no bearing tree has been observed to recover from infection. Other inoculation experiments and also field observations indicate that apple trees are more susceptible to this root disease after they begin heavy bearing than before. This young bearing stage in orchard trees has also been found to be an especially susceptible period for winter injury and for such diseases as Phytophthora collar blight and *Xylaria* root rot.

Repeated cases have been found showing the ability of this disease to kill trees very rapidly. In the summer of 1936, some trees that had made good terminal growth the previous year died while they were carrying a good load of fruit. In another case an apparently vigorous 15-year-old tree was well-laden with mature and marketable fruit the latter part of August, even though it was completely girdled by the fungus. In both instances the action of the pathogen was so rapid that the appearance of the tops gave little indication of the diseased condition of the roots until the trees died.

The pathogenicity of this fungus also was studied by isolation and inoculation work. Cultures used for inoculation were obtained from spores and from isolations made from diseased apple tree roots taken from the margin of healthy tissue. Work with this fungus has demonstrated what has also been observed with other root rot organisms—that the quantity and types of inoculum used influence infection. The best type of inoculum was obtained by growing a pure culture of the fungus a month or two on short sections of heat-sterilized apple twigs. Where the tree was inoculated *in situ* root tissue was exposed by removing the soil, the inoculum placed against the uninjured root and the soil immediately replaced. The trees were not further disturbed until the end of the growing season, when they were inspected for infection. When trees were inoculated while in storage the inoculum was held against the root with a rubber band.

Young trees growing in the nursery were successfully inoculated with naturally infected apple roots and also with a pure culture of the fungus. In September, 1936, naturally infected roots were placed beside 10 3-year-old trees in a nursery row, and a year later 5 trees were infected, showing typical signs of the disease. In 1937 and 1938 4 inoculation experiments were made on young trees growing *in situ*, using a pure culture of apple twig inoculum. In one experiment a culture obtained from spores was used and in the other

3 the cultures were from diseased apple roots. All 4 cultures produced the disease, averaging 18 per cent infection in 49 inoculations. A like number of checks were uninjected.

The pathogenicity of the fungus with respect to dug apple trees also was studied by inoculation experiments. In April, 1937, 40 1-year-old seedlings from the nursery storage were inoculated by binding without wounding a twig culture of the fungus with a rubber band to the main root. The trees were then immediately planted. An equal number from the same lot of trees were planted without inoculation, to serve as checks. By July 20 the roots of all the inoculated trees were dead, while the checks were unaffected and grew in a normal manner. From the infected roots was isolated a fungus that appeared to be identical with the fungus used for inoculation.

In March 1938, apple seedlings were divided into 3 comparable lots of 25 trees and each lot inoculated with 1 of 3 different spore isolates. These seedlings were then stored in a cool cellar in peat, and, on June 1, examination showed an average of 95 per cent infection on all 3 lots. Root cuttings inoculated and planted in February, 1938, showed 9 infections in 18 inoculations by October of the same year, while the noninoculated checks showed no infection.

These experiments indicate that young trees, as they grow in the nursery, may be successfully inoculated with a pure culture of the pathogen but the percentage and degree of infection obtained on such trees have been very much less than on stored nursery trees or trees disturbed by digging.

#### THE FRUITING STAGE OF THE FUNGUS

According to Burt<sup>4</sup> *Corticium galactinum* (Fr.) Burt, fruits on a variety of substrata, including wood of both coniferous and broadleaf species, is widely distributed in North America, and is present in the West Indies and Japan. It seems reasonable to expect the species so defined to be composed of several physiologic strains, but the writers have studied only the form on apple roots.<sup>5</sup> It is hoped that a consideration of the broader aspects of the species will eventually be undertaken to determine to what extent native trees and shrubs, also, when growing under natural conditions, are affected by it.

In general, the fruiting on apple roots and stumps agrees fairly well with the description given by Burt, except that he does not mention the conspicuous slightly protruding paraphyses (Fig. 1, A), which have been observed in all our specimens. Most sporophores collected do not contain a distinct basidial layer; therefore, these paraphyses constitute the most characteristic feature of the species. Since no illustrations of the fungus were given in Burt's monograph of the genus, drawings are given showing some characters of the organism dealt with in this paper.

<sup>4</sup> Burt, E. A. The Thelephoraceae of North America XV. Ann. Missouri Bot. Gard. 13: 173-354.

<sup>5</sup> A sporophore from an apple stump was sent to L. O. Overholts, who identified it as *Corticium galactinum*.

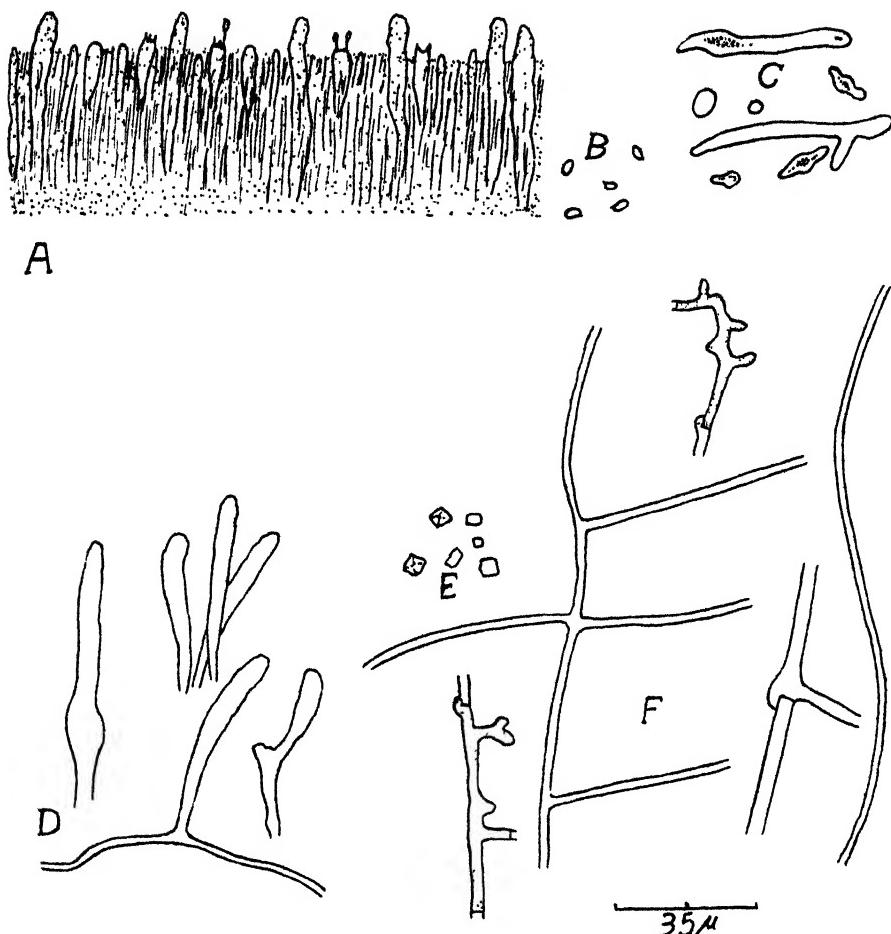


FIG. 1. *Corticium galactinum*. A. Sketch of a section through the hymenium. B. Basidiospores. C. Germinating basidiospores. D. Paraphysis-like bodies from a culture. E. Crystals from pure cultures on malt agar. F. Hyphae from pure cultures.

The dense white to light-cream buff layer of mycelium, on which the hymenium develops when conditions are favorable, may persist over a period of several years on old apple stumps and roots. The basidia apparently develop during damp weather in summer or fall and soon collapse. They are entirely absent under dry conditions. In fact, sections from fresh sporophores, which gave good spore prints, contained very few mature basidia. Sporophores usually are formed in soil cavities about rocks or on roots (Fig. 2, D), but during favorable growing conditions the fungus may grow out over the surrounding soil and debris where it fruits in abundance. It may be that the habit of fruiting on soil and surrounding debris is responsible for its having been reported on such a variety of substrata.

The dry fruiting layer is white to light buff; but in a damp and sporulating condition it has a slightly waxy appearance on the surface and, in



FIG. 2. A. Mycelium of *Corticium galactinum* on the surface of an apple root. This photograph also shows the sharp demarcation between sound and diseased tissue indicated by the arrow. B. Infected blackberry showing hymenium of *C. galactinum* around the collar and mycelium on the roots. C. Infected apple root covered with thick weft of mycelium. D. Hymenal layer on an old apple root.

color, ranges from light buff to ochraceous buff.<sup>6</sup> Von Schrenk<sup>7</sup> states that the fruiting bodies are "bright red orange leathery sheets" but our observation indicates the hymenium is not so highly colored. The thickness of the fruiting body presumably depends upon its age; specimens several years old have been observed with fruiting bodies up to 500  $\mu$  thick.

The structure of the sporophore is not very distinctive, except for the paraphyses, which project up to 12  $\mu$  above the surface of the hymenium and penetrate into the subhymenium to a depth of about 30 to 40  $\mu$ . Basidia with immature spores attached may be found occasionally in sections of fruiting sporophores (Fig. 1, A), but usually even young basidia are difficult to find. Spores are not abundant on dry specimens but are easily obtained from good fresh material. A spore print can be obtained by placing under a bell jar or other closed container such sporophores with the hymenium downward. Also, small sections 1 or 2 cm. square, cut from the hymenial layer and suspended over Petri dishes containing nutrient agar, will deposit spores in great abundance for a period of 12 to 18 hours if left in a moist condition. The mature basidiospores are ovoid, hyaline, smooth, and 3-4 by 2-3  $\mu$  (Fig. 1, B) in size.

#### HOST RELATIONS

The white root-rot organism will attack the roots of plants other than the apple, but the observations to date have included only plants growing in the vicinity of the focus of inoculum of diseased apple trees, or, in one case, an oak stump. The pathogen has been found growing abundantly on the roots of blackberry (*Rubus allegheniensis* Porter), dewberry (*Rubus flagellaris* Willd.), Japanese wineberry (*Rubus phoenicolarius* Maxim.), dogwood (*Cornus florida* L.), sumac (*Rhus glabra* L.), and white campion (*Lychnis alba* Mill.). More information is needed concerning the susceptibility of various species.

One instance has recently been found in which ornamental plantings have been affected by this disease. In a yard near Hyattsville, Maryland, two disease spots were observed in which a young holly tree, a dogwood, and two *Kalmia* bushes were removed because of this disease. Near the spots where these plants died was an oak stump on which *Corticium* was growing and producing fruiting bodies typical of *Corticium galactinum*. This and other observations indicate that when ornamental shrub plantings are made in newly cleared land, white root rot may become a problem.

#### THE FUNGUS IN CULTURE

##### Isolation and Spore Germination

Spores deposited directly from segments of the hymenium, as described above, on Petri dishes containing Difco cornmeal agar or malt agar germinated in 10 to 16 hours (room temperature of about 26° C.) (Fig. 1, C.)

<sup>6</sup> Ridgway, R. Color Standards and Color Nomenclature, 43 pp. Washington, D. C. 1912.

<sup>7</sup> See footnote 1.

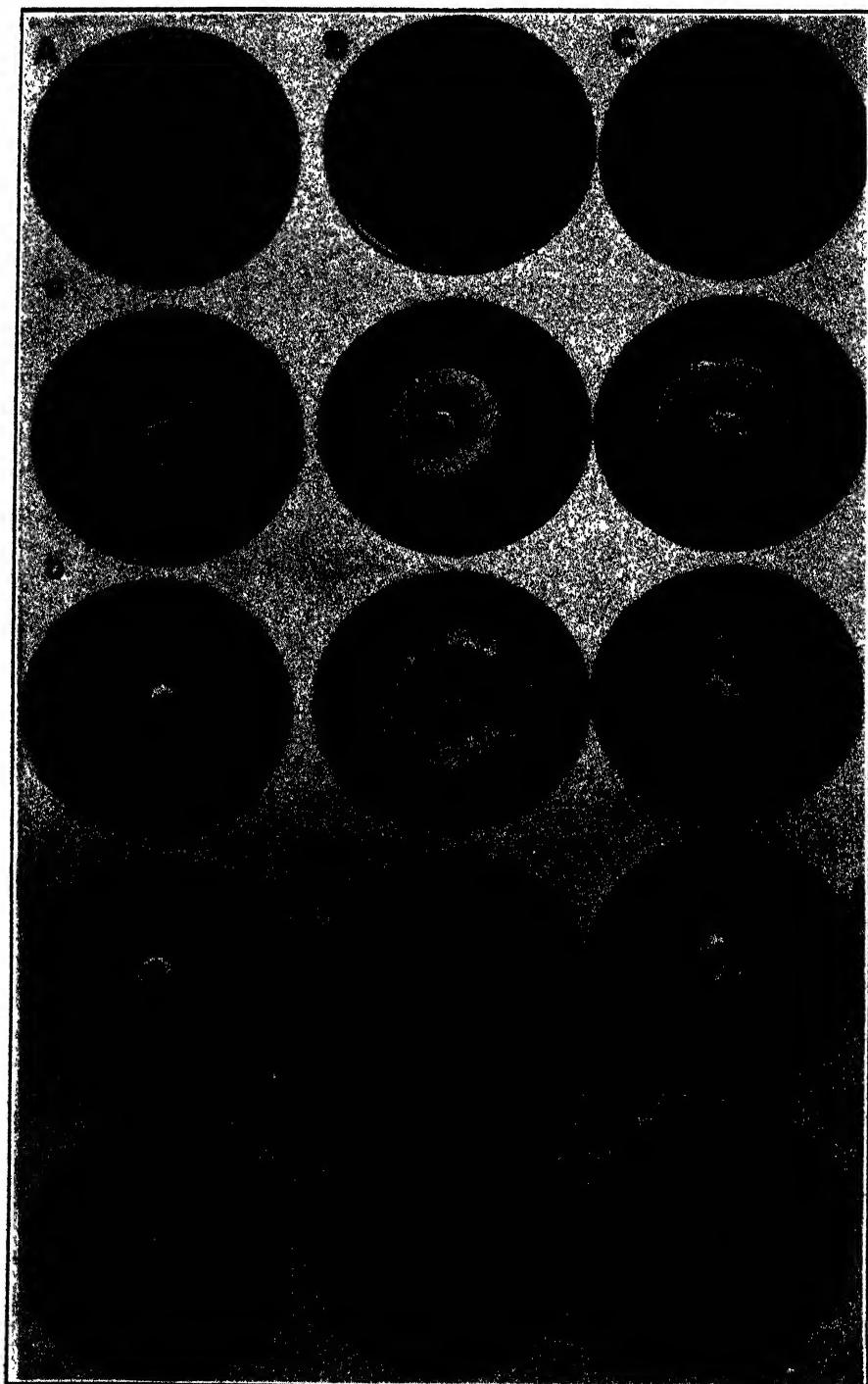


FIG. 3. Eight-day-old cultures of *Corticium galactinum* grown in constant temperature chambers. A. Culture from diseased apple root. B. and C. Basidiospore cultures. a. 34° C., b. 31° C., c. 25° C., d. 20° C., e. 15° C.

and a large proportion of the spores were viable. Cultures were obtained also from recently formed lesions on living roots and were similar in general growth characteristics, as well as in microscopic characters, to those from spores.

#### Temperature Relations

Four cultures obtained from spores and 2 from diseased roots were grown on 2.5 per cent malt-agar medium in the dark at various constant temperatures. The average diameters of mycelial mats for all cultures, including culture 7138-S after 1 day at ordinary room temperature followed by 7 days in the constant temperature chambers, were as follows: At 10° C., 16 mm.; at 15°, 22 mm.; at 20°, 41 mm.; at 25°, 61 mm.; at 31°, 45 mm.; at 34°, trace; and at 40°, no growth (Fig. 3). Growth rate at the various temperatures was fairly uniform for all cultures except spore culture 71383-S, which had a mat diameter of only 26 mm., at 20°; 41 mm., at 25°; and 33 mm., at 31°. The optimum temperature for growth of all cultures used

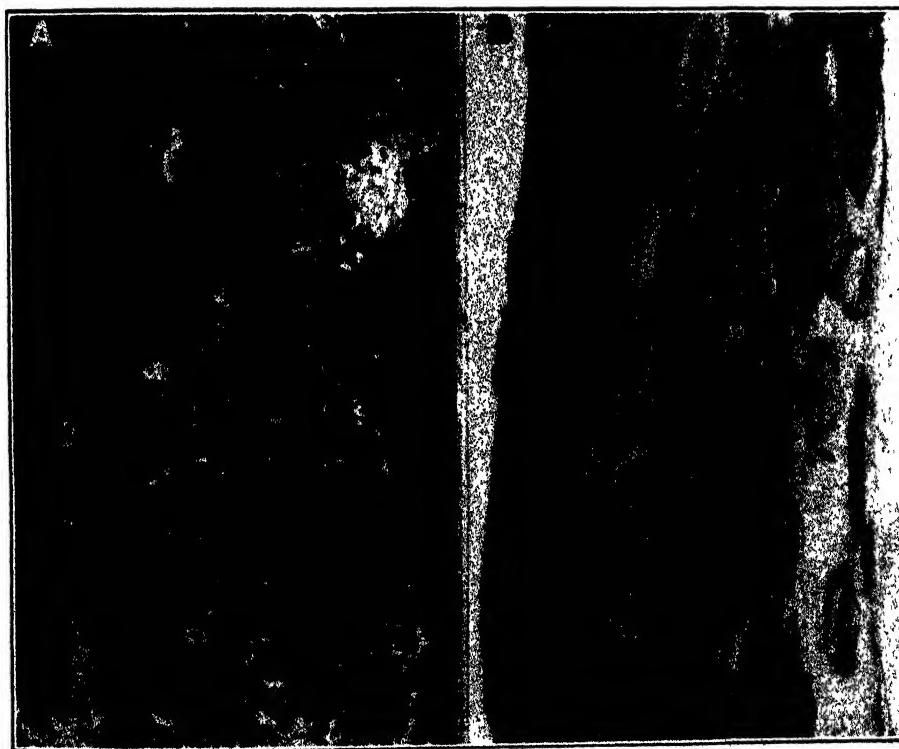


FIG. 4. A. Distorted apple root illustrating partial recovery. The depressions shown at the margin of the photograph indicate areas that were killed by *Corticium galactinum*. B. Dead apple root with bark removed to show the characteristic rings formed around areas of initial killing. The entire root was killed before callus tissue was formed.

was between 25° and 31° and maximum temperature was apparently slightly above 34°.

## SUMMARY AND CONCLUSIONS

The paper describes a white root rot of apple trees that escaped the notice of pathologists from its discovery in 1902 until 1932. The fungus isolated from diseased roots has been identified as *Corticium galactinum* by comparing it with cultures obtained from sporophores of *C. galactinum* from other sources. The cultural characteristics of the fungus are described.

While the disease has been found in relatively few orchards scattered over Delaware, Virginia, Maryland, Tennessee, and Indiana, more surveys will probably show its distribution to be much more widespread than is now known.

Since the disease has as yet been observed only in orchards set on new land or adjacent to woods, it seems probable that new land furnishes conditions favorable for the supplying and the maintenance of the inoculum. This observation, together with the virulence of the disease in certain orchards, justified the conclusion that consideration should be given to this disease in choosing an orchard site.

The organism is very destructive in its attack. The spread from tree to tree seems slow but after infection is established killing is very rapid. A slow advance of the disease has been noted in all the orchards under observation.

Young apple trees were successfully inoculated with the fungus described in the paper. Undisturbed nursery trees were much less susceptible to the disease than dug trees. Trees of bearing age showed greater susceptibility to the disease than younger ones.

Several other species of plants when growing in proximity to diseased apple trees or oak stumps became infected.

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APPLE DIEBACK IN CALIFORNIA<sup>1</sup>

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(Accepted for publication September 9, 1939)

## INTRODUCTION

Dying back of apple branches usually is accompanied by relatively few distinctive characteristics. The disorder here presented is commonly prevalent in the Sebastopol area of Sonoma County, California, one of the two leading apple districts of the State, and has been seen in at least one other county (Eldorado). Trees may develop severe symptoms for the first time at any age from 1 or 2 up to 25 years or more (Fig. 1, A, D). Depending on the severity, the buds may die without starting growth, may push out a few

<sup>1</sup> Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

small leaves and then die, or may slowly develop a sparse foliage of small, narrow leaves (Fig. 1, B). When the buds die early, the bark above the ground may break down in a few weeks, often with a strong odor, which has given rise locally to the term sour sap. In somewhat less severe cases, the bark becomes densely covered with protuberances, each underlaid by necrotic tissue in the interior of the bark. These may break down later, often with more or less concentric marking. This bark symptom falls in the general class variously called measles, target canker, etc.

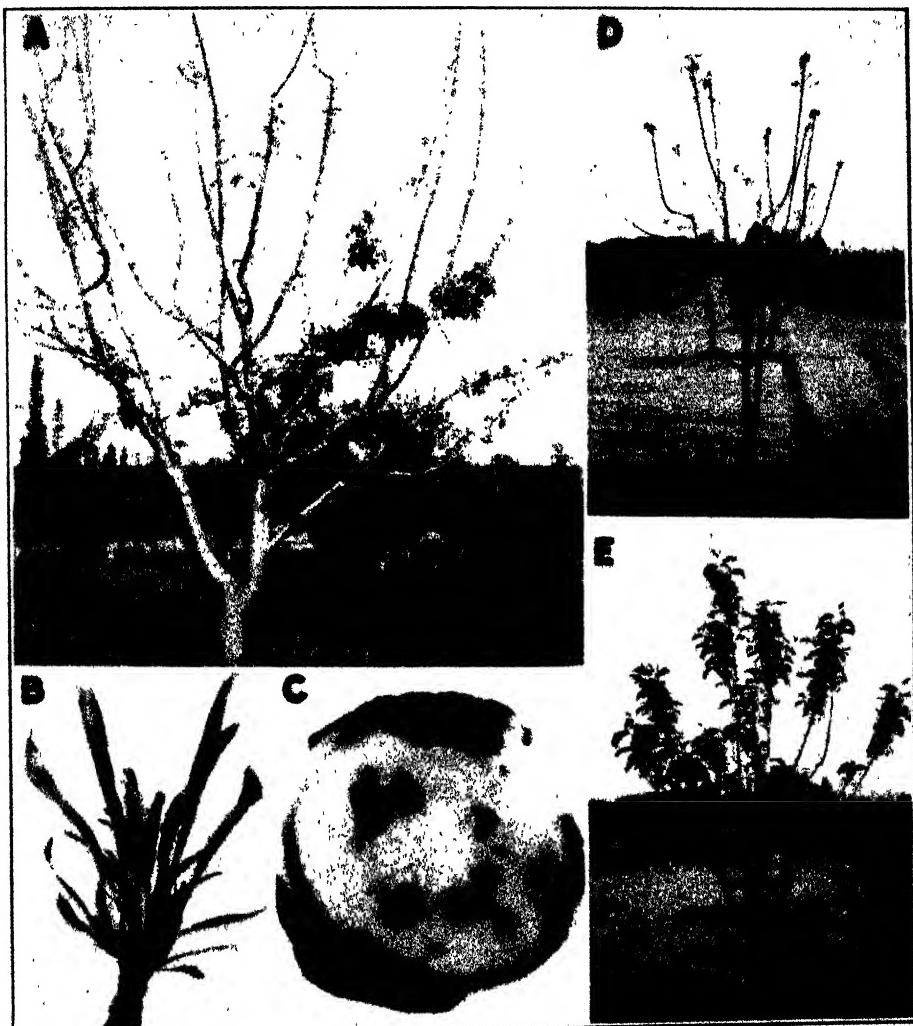


FIG. 1. A. Gravenstein apple tree showing dieback symptoms. B. Dieback disorder on a Spitzenberg apple tree. Opened buds had very narrow leaves and short petioles. C. Cross section at the base of a bud that failed to develop in the spring and remained dormant all summer. Note the necrotic pockets in the region of the vascular elements. X15. D. Young Rome Beauty tree showing severely affected buds. E. Apparently healthy Rome Beauty tree adjacent to that in D photographed at the same time, May 5, 1930.

Much of the disorder is found in Gravenstein, which is by far the leading variety of the Sebastopol area, but Spitzenberg and Wagener seem to be distinctly more susceptible. Delicious is particularly susceptible to the measles type of trouble here as elsewhere. Pears are affected with symptoms similar to those of the apple.

Specific fruit symptoms were seen in 1937, apparently for the first time in California; though the dieback phase of the disease has been under observation for 30 years or more (19). These fruit symptoms (Fig. 2) are in the main typical of the cork and drouth spot, adequately described by Mix (17) and others (1, 2, 3, 4, 13, 14, 18), and appeared following heavy winter rains and high early summer temperatures.

Rosette or little leaf, which also occurs in the Sebastopol area (6), is distinguishable with difficulty, if at all, by symptoms from the dieback disease under consideration. Usually, however, on rosette trees the tips of many branches remain alive until late stages with distinctly thickened shoots and very short internodes near the tips resulting in the compact tufts

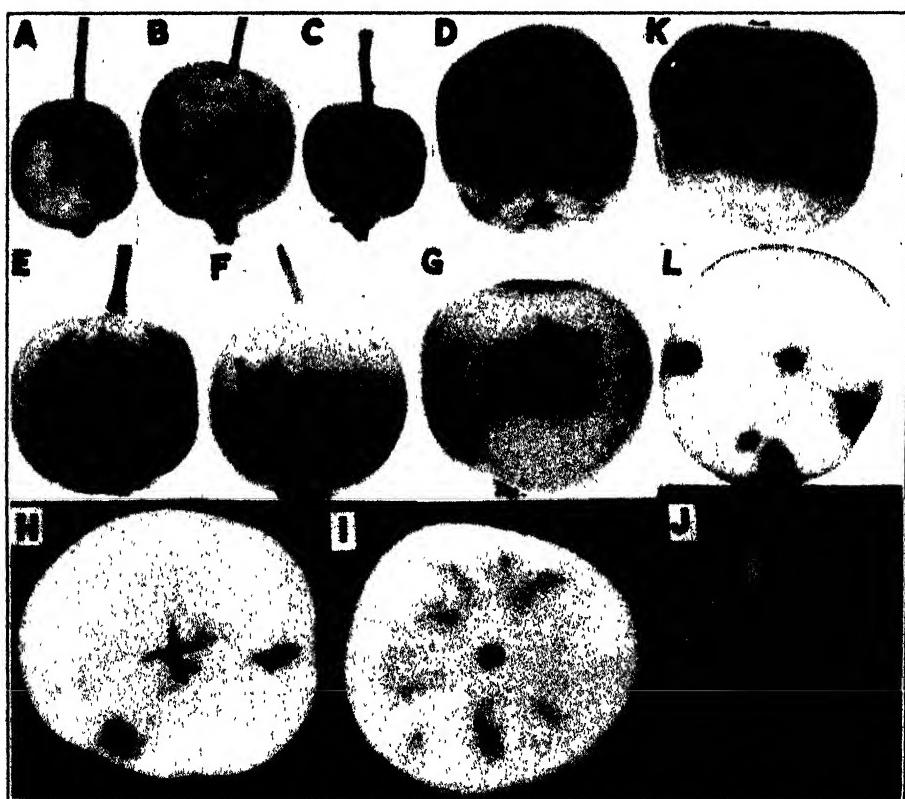


FIG. 2. A-D. Symptoms of drouth spot on fruits of Spitzenberg apples. E-G. Symptoms on fruits of Red Rome apple. Note the fingerprint pattern in F and G. H-J. Internal cork on a Gravenstein apple. H. Internal light-brown areas, which usually occur between the large vascular elements. I and J. A diffused type of cork. K. External appearance of fruit from a Jonathan apple tree affected with internal cork. Note pitting of the fruit. L. Cross section of the fruit shown in K. All  $\times \frac{1}{4}$ .

of leaves, which gave rise to the name. The petioles here are often almost entirely suppressed, while on dieback trees they usually reach a length of at least half an inch or so. No specific fruit symptoms seem to have been found on rosette trees.

#### SOIL OF AFFECTED ORCHARDS

Orchards in which dieback occurs are planted in Goldridge fine sandy loam soil. The subsoil of the better orchards is typically a pervious clay, while in many, if not all, of the affected areas the subsurface strata contain considerable cementing material. This soil is distinctly acid, relatively low in fertility, especially K and P (12), and is dependent upon winter rainfall (15 to 40 inches) for its water supply. Glass-electrode determination of pH on soils from very severely affected orchards ranged from 4.95 to 5.45 (top soil). The application of chicken manure is commonly practiced among the apple growers in this district.

#### EXPERIMENTAL

##### Bacteriological Approach

Numerous attempts to isolate a pathogenic organism were made by different individuals at the California station at different times, which yielded only negative results.

The idea that some toxic material was being liberated by soil micro-organisms or was being formed by some unfavorable soil condition has been entertained for some time. It was observed that soil samples from affected orchards, when artificially waterlogged and subsequently incubated anaerobically, always had a putrid odor, were dirty blue, and the soil filtrate proved toxic to barley seedlings and to young, tender apple shoots. Apple seedlings, grown in artificially waterlogged soil that had been incubated anaerobically in the laboratory for a month, developed very poorly in comparison with those grown in soil that was not waterlogged but was also incubated under anaerobic conditions for a month. However, the plants in the treated lot eventually recovered unless the soil was constantly waterlogged. It is quite possible that microorganisms may cause the dieback condition by absorbing from the soil large amounts of elements that are indispensable for the normal development of higher plants. By Cholodny's method (7), applied to the rhizosphere of diseased apple trees, it appeared that long and short bacterial rods predominated over fungi and *Actinomyces*, while, in the rhizosphere of healthy (unaffected) trees, fungi and *Actinomyces* are more abundant. Sterilization of soil from a dieback orchard either by steam or with formaldehyde failed to produce symptoms of boron deficiency in annual plants, symptoms appearing after inoculation of sterilized soil with a small quantity of nonsterilized soil and subsequent incubation. More detailed treatment of this phase will appear in a later publication.

### Relation of Boron to Dieback

In the spring of 1937, in orchards where dieback of apple was severe, morning-glory plants were found with dead or dying growing points, burning of tips of the leaves, and abscission of blossoms. Also, one sunflower plant appeared stunted in growth, with browning and malformation of the developing leaves. The plants were taken to the greenhouse for observation, and it soon became apparent that these plants were suffering from boron deficiency. By adding 1 mg. of boric acid to a gallon of the same orchard soil in which these plants were found, normal growth of the morning-glory and sunflower plants was resumed.

One-gallon tin cans, painted on the inside with asphaltum, were filled with either top soil or subsoil taken at a depth of 2 feet. To one series of 10 cans in each case, 1 mg. of boracic acid was applied at the time of planting, while the second series had no treatment. All sugar beet, sunflower, nasturtium, and lettuce plants grown in the nontreated soil developed characteristic symptoms of boron deficiency, while in the treated lots all plants developed normally. The low availability of boron in soils of affected apple orchards, as indicated by these tests, suggested that application of boron-containing compounds to the diseased trees might prove beneficial. The use of boron as a corrective for similar conditions in apples has been made by a number of investigators (3, 5, 15, 16, 18).

It is interesting to note that the total sugar analysis of the leaves from the diseased and healthy apple trees employing Hassid's (9) method showed 5.56 per cent in leaves and 2.6 per cent in twigs for diseased and 3.8 per cent and 3.04 per cent for healthy trees. This seems to be in accordance with the statement of Haas and Klotz (8) that sugars accumulate in the leaves whenever boron is omitted.

Attempts to produce symptoms of boron deficiency in deciduous fruit trees were made by growing apple, peach, and apricot plants in subsoil obtained from affected apple orchards and watering them with distilled water. Some of these plants were seedlings and others were commercial varieties grafted onto seedlings. Only apricot developed symptoms of boron deficiency, as described by Hoagland, Chandler, and Hibbard (11), this being corrected by giving 0.1 g. boric acid to each jar containing 10 kg. of nonsterilized subsoil.

Field application of boron to diseased trees was started in the winter of 1936 and the spring of 1937. Boric acid or borax was applied to the trees by boring a hole in a large branch or in a trunk and packing it with the material. These treatments produced no striking difference in the treated trees in comparison with nontreated trees.

In the fall of 1937 and in January and February of 1938, 35 trees in one orchard (Gravenstein, Delicious, Jonathan, and Rome Beauty) having dieback of branches, cork, and measles, and 20 trees in another orchard (predominately Spitzemberg) showing typical symptoms of drought spot and dieback, were treated by broadcasting borax on the soil around the trees

within a radius of 3 feet from the trunk. In one of the treated orchards, the trees appeared more thrifty the following year than the nontreated. One large Gravenstein tree with very severe dieback symptoms was greatly improved the year after receiving 10 pounds of borax, and still better in the second season. Mild dieback and severe and moderate cork symptoms of 8-year-old Jonathan trees were no longer visible after 2 pounds of borax had been applied as dressing, while corresponding checks were badly affected. The fruit on large Gravenstein apple trees still had cork when treated with  $\frac{1}{2}$ , 1, 2, 3, and 4 lb. of borax. Cork was considerably reduced in such trees treated with 5 lb. or more of borax.

In the other orchard, where drought spot on fruit was abundant the season prior to treatment, no conclusion could be reached, since fruit symptoms were lacking and the general appearance was similar for all trees.

Delicious trees, affected with measles in the first-mentioned orchard, seemingly were benefited by the boron treatment, since they made good growth and did not show the protuberances and cracking of bark on the new growth.

On the basis of somewhat limited treatments, there seems to be a definite indication of beneficial effects by applying boron to trees affected with cork and measles and to some trees showing dieback symptoms only.

#### RELATION OF POTASSIUM TO DIEBACK

Chemical analyses of soils in affected orchards in the Sebastopol area have shown a very low potassium availability, according to the work of Hoagland and Martin (12). Hill and Davis (10), in Canada, found cork in orchards grown on soils with low available potassium. In 1937, McLarty, Wilcox, and Woodbridge (16), in referring to orchards with dieback, stated that "heavy applications of potash have in some cases materially lessened the disease symptoms." At the suggestion of W. H. Chandler, a few severely affected trees were treated with heavy applications of potassium sulphate (approximately 25 and 75 lb. per tree) early in 1937. The treated trees improved considerably in one orchard and only slightly in another. It is possible that the boron in these rather large amounts of potassium sulphate may have produced the observed effect. The experience thus far on the whole, however, suggests that in some, if not all, of these orchards, more than one element will be required to completely cure the affected trees. At least it seems to be true that if boron alone is to be completely effective it must be used in much larger amounts than is required in other areas. More extensive experiments are under way including combination treatments with boron, potassium, and other materials.

#### SUMMARY

Dieback of apple trees, often accompanied by a type of "measles" and occasionally by cork and drouth spot of fruit, is prevalent in the Sebastopol area of California.

The soil is distinctly acid and low in available nutrients, notably potassium.

Such annual plants as nasturtium, sugar beet, and sunflower, grown in soil from affected orchards, developed boron-deficiency symptoms curable by addition of small amounts of borax or boric acid to the soil.

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# METHODS OF VALUE IN BREEDING AUSTRIAN WINTER FIELD PEAS FOR DISEASE RESISTANCE IN THE SOUTH<sup>1</sup>

J. L. WEIMER

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## INTRODUCTION

Soon after the writer began the study of the diseases of Austrian Winter field peas (*Pisum arvense* L.) at the Georgia Agricultural Experiment Station, three major difficulties were encountered. In the first place, almost none of the varieties of peas (*P. arvense* and *P. sativum*), except the Austrian Winter, survived the usual winter temperatures. This made it impractical to test any large number of varieties of peas for disease resistance under field conditions during the winter. Plants from seed sown in the spring were killed very early by insects and diseases; hence, changing the planting time from autumn to spring was of no assistance. Even though some varieties survived the milder winters, the plants usually succumbed to diseases or insects, or both, before seed matured. Furthermore, nature could not be depended on to produce an epiphytotic of the diseases being studied, so that the relative resistance of varieties, plants, or hybrids could be determined. This made it necessary to use some method of artificial inoculation.

Solutions for certain of these difficulties have been found and are described in this paper. No claim is made that these methods are new, but rather that they have been useful in solving the problems under consideration, and it is hoped will be of assistance to others having similar problems.

## ELECTRIC HOTBED

Since so few varieties of peas survived the winters at Experiment, Georgia, or even at Tifton, the following method for overcoming this difficulty was tested.

Two hundred feet of electrically heated and controlled hotbed in 50-ft. units, each 5½ ft. wide, were constructed (Fig. 1, A). The walls of the beds were made of pine boards and were 1 ft. high on one side and 2 ft. on the other. The high side was covered with heavy building paper primarily as protection over the crack between the boards and at the corners and around knot holes. Instead of sash, the top was covered with a heavy cotton sheeting. The covers were used only on nights when there was danger of frost and during the coldest days. Thus, for all practical purposes, the plants were exposed to field conditions, except during the coldest weather.

<sup>1</sup> Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 65, Journal Series, Georgia Agricultural Experiment Station.

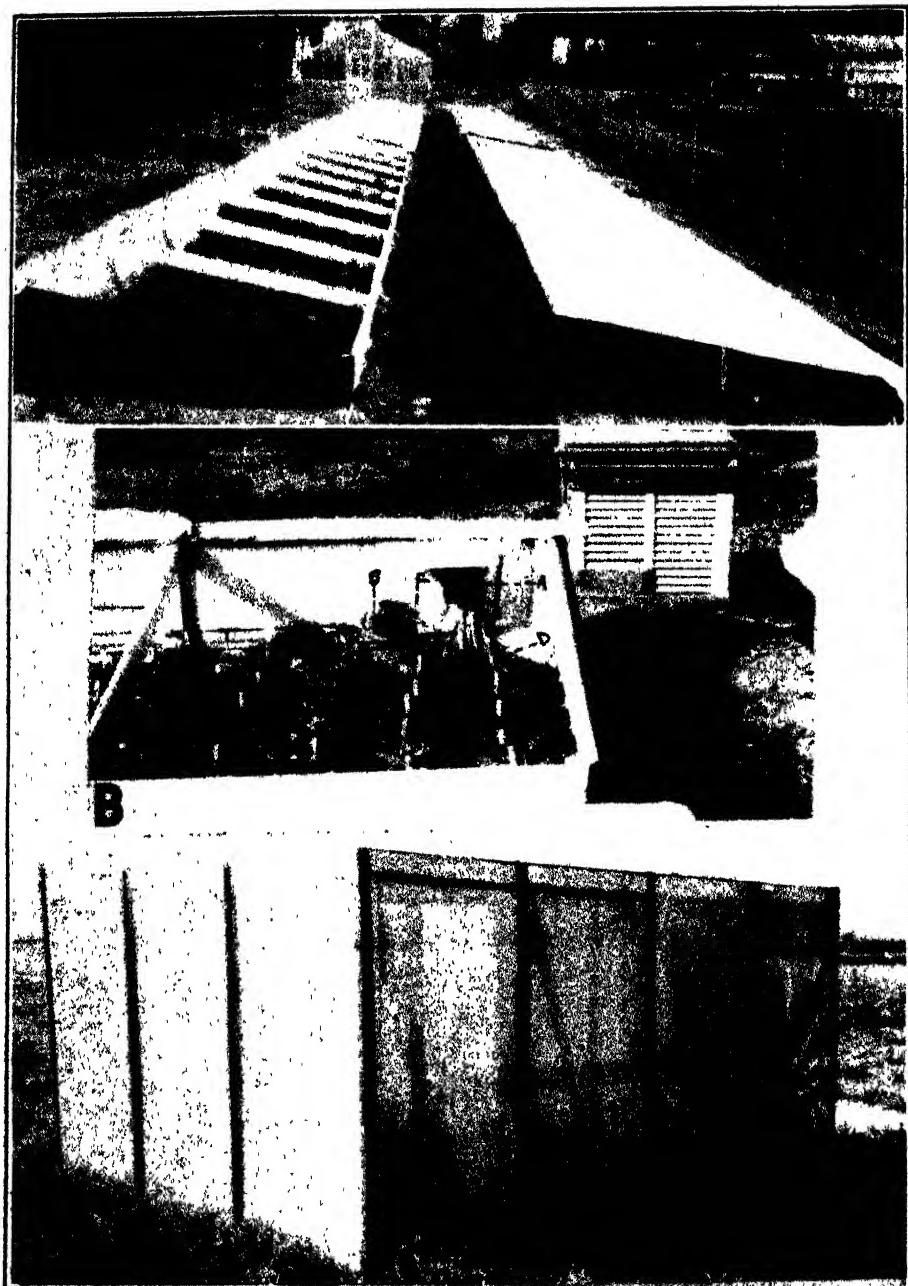


FIG. 1. A. Four hotbeds each 50 feet long by 5½ feet wide, used to grow peas for testing for disease resistance. First bed on the left has the cloth cover rolled back; the other 3 have the covers in place. Several details of construction are shown here and also in B. B. A cut-off switch A (inked letters), thermostat B, and thermostat bulb C are shown. The thermostat bulb was suspended over a pot. The thermograph bulb D was placed over a pot also so as to obtain the temperature to which the bases of the plants were actually exposed. C. A cloth-covered house in which a good seed crop has been obtained from a number of different varieties of peas.

The electric cable was placed on the surface of the soil and looped back and forth across the bed at distances convenient for spacing rows of pots, in which the plants grew. Thus the cable lay along two sides of every pot (Fig. 2). At first, 360 ft. of cable were used for each 50 ft. of hotbed, but later 120 ft. more were added to 2 of the beds. This additional cable was not needed, since the lowest official temperature for the winter was only 19° F. One thermostat was used for each bed (Fig. 1, B). Two hundred and twenty-volt current was utilized. The thermostats were set so that the heat was turned on at about 35° F. The thermostat markings were not reliable, but by placing the bulbs in water at the desired temperature, the settings could be made readily. Soil-thermograph bulbs placed on the tops of pots showed the temperature to which the bases of the plants were subjected. None of the plants were frozen, excepting a few leaves, which had grown up against the cloth top. Judging from the thermograph records (Fig. 3), it seems probable that the plants would have survived a temperature several degrees lower, but the limit is not known.



FIG. 2. A closer view of a section of one bed than shown in figure 1, A. Here the arrangement of the cable with respect to the pots is shown. The 3 longitudinal strands of cable were added after the season was partly over but were not needed.

This would depend, of course, on the hardiness of the plants, as well as whether the low temperature was accompanied by a high wind, which would tend to dissipate the heat rapidly and to displace the covers. Additional protection could be provided readily by using a heavier cloth or by installing more cable. The beds held 354 6-inch pots each. As many as 10 seeds were planted in a pot, hence a considerable number of plants can be tested by this method.

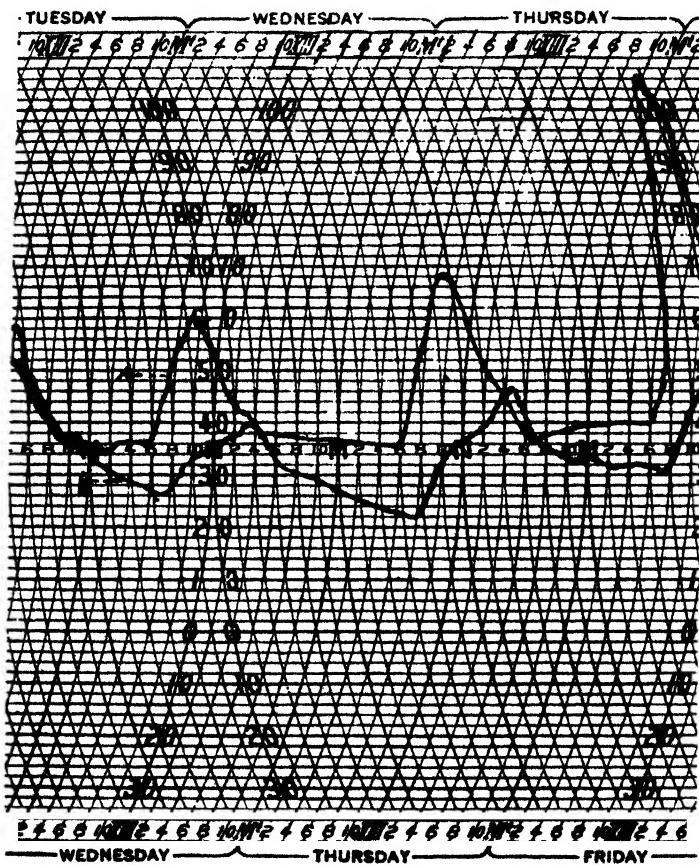


FIG. 3. A section of the thermograph record covering the period of Feb. 21 to 23, 1939. The curve A shows the variation in the temperature in the hotbed and curve B that of the air outside. These curves show that on Wednesday morning, Feb. 22, the air temperature was approximately 26° F., while that of the hotbed never went below 34° F., and that the air temperature the next night reached a minimum of 22° F., while that in the bed hardly reached 35° F. The high (maxima) for the days are not significant. The temperature in the beds under the cloth went up rapidly as soon as the sun reached them. In some instances the sun also shone directly on the thermograph bulb for a short time after the cloth was removed.

#### GROWING SEED

Attempts to grow seed of any lot of plants that showed some disease resistance or other desirable character and to breed peas were greatly handicapped by the fact that most varieties of peas failed to set and mature

seed under field conditions at Experiment. Peas planted in the greenhouse and given additional light from 5 to 11 p.m. produced seed fairly well during the winter months. Greenhouse space, however, was insufficient to carry on the work on the scale desired; hence, a cloth-covered shelter was tried. It was known that similar shelters had been used successfully by other workers both for sweet and English peas. Austrian Winter peas, however, appear to be more exacting in their requirements for seed production than many of the field and garden varieties. A shelter  $9 \times 12 \times 6$  ft. was constructed of  $2 \times 4$  in. lumber. This frame was covered with a good grade of cheesecloth, which kept out all but the smallest insects. The door was of the ordinary type, such as is commonly used on insect cages (Fig. 1, C). Two 500-watt Mazda lamps with 18-in. R. L. M. Dome reflectors were so attached to the ceiling as to provide approximately equal illumination for all of the plants. These lights were turned on at 5 p.m. and off at 11 p.m.

Two crops of plants have been matured under cloth in this manner, one in the spring and one in the autumn. The spring crop consisted of plants grown from seed sown in pots in the autumn and held in the hotbeds until March 30, when they were removed to the cloth shelter. All varieties used had matured seed by June 6 or earlier. The autumn crop was planted in pots on July 29, 1938, and most varieties had matured their seed by November 15. Thus it is possible to grow two crops a year. In fact, the seed from the spring crop can be made to mature a seed crop early enough to be planted in the field or hotbed in the autumn.

#### METHOD OF INOCULATION

Early attempts to produce heavy infection of Austrian Winter peas with *Ascochyta pinodella* and *Mycosphaerella pinodes* under greenhouse conditions gave unsatisfactory results. A few small lesions were produced on the leaves and stems but these were not sufficiently numerous or large to appreciably damage the plants or enable one to judge their relative resistance.

The method now in use makes it possible to produce a severe epiphytotic of the diseases under outdoor conditions. The plants to be tested were potted and held in the hotbeds described above. Giant cultures of the fungi being tested were cultured on Austrian Winter field peas. The dry peas were soaked over night in water and boiled a few minutes the next morning. The water was then drained off and the peas were put in flasks or 2-quart fruit jars, plugged with cotton, and autoclaved at 18 pounds' pressure for 1 hour. Inoculation of the flasks was accomplished by adding several cc. of a suspension of spores from oat-agar cultures in sterile water and shaking the flasks vigorously every day or so to spread the inoculum. After about 10 days the pea medium was covered with spores. A heaping teaspoonful of these spore-coated peas was scattered over the surface of each pot beneath the young seedlings. The pots were then forcibly sprinkled with water so as to spatter the spores upon the young plants. After inoculation the cloth covers were kept over the hotbeds for 48 hours.

During this time the cloth was wet thoroughly several times to help maintain a high humidity in the bed. A second inoculation was made 11 days after the first. The seed was planted October 15, 1938, and the inoculations were made on November 11 and 22. By December 7 a few plants of the most susceptible varieties were nearly decayed off at or near the soil surface. At this time the amount of infection varied greatly; but by March 1, 1939, there was a severe epiphytic in all the beds. The spores, splashed from the original inoculum as well as from the lesions fruiting on the stems and leaves during watering and by the rains, produced an increasingly heavy infection as the season progressed. Eventually, many plants were killed, large areas of the stems of others were blackened, and the leaves of most of the varieties were more or less severely spotted.

#### SUMMARY

The use of an electrically heated and controlled hotbed to prevent the freezing of English peas under test for resistance to *Ascochyta pinodella* and *Mycosphaerella pinodes* during the winter and spring months at Experiment, Georgia, is discussed.

Austrian Winter and many other field and garden peas do not set seed consistently or in satisfactory quantity in the field in many parts of the South. These crops will set seed out of doors under cheesecloth. At certain times of the year it is necessary to give the plants additional light. This was done by the use of 500-watt lamps alight from 5 to 11 p.m. each day. Two crops of seed a year can be grown by this method.

An epiphytic of the diseases under investigation was produced by inoculating hotbed-grown potted plants with cultures of the pathogens.

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#### A DRY ROT OF POTATO STEMS CAUSED BY FUSARIUM SOLANI<sup>1</sup>

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(Accepted for publication August 19, 1939)

Potato plants showing symptoms unlike those of any of the common potato diseases were observed by the author in a test plot of Bliss Triumph potatoes located at Scottsbluff, Nebraska, in 1935. The most noticeable symptoms were yellowing and wilting of the foliage, sometimes preceded by a rosetting of the top and the formation of aerial tubers in the leaf axils. The underground stem always showed some rotting, varying from a slight basal rot to a complete dry rot characterized by a shredded appearance due to the remaining strands of woody tissues. In the less advanced stages the rot produced a softening of the pith, whereas the vascular cylinder ap-

<sup>1</sup> Published with the approval of the Director as Paper No. 236, Journal Series, Nebraska Agricultural Experiment Station.

The writer is indebted to Mitrofan Afanasiev, James H. Jensen, and W. E. Deacon for assistance in this investigation.

peared woody and light yellow. The roots of affected plants were severely rotted. Neither stolons nor tubers were directly affected. These plants were at first thought to be affected with *Fusarium oxysporum* Schlect., or even *F. solani* (Mart.) App. and Wr.) var. *eumartii* (Carp.) Wr. (Syn. *F. eumartii*) with atypical symptoms resulting from conditions favoring a rapid decay of the stem, such as sometimes occurs in irrigated fields. Later in the same season, however, similarly affected plants were observed in non-irrigated fields under very dry conditions. Plants were received also from North Dakota showing similar symptoms.

A large number of isolations were made from the underground parts of many of these plants. Cultures of *Fusaria* were obtained from all specimens and 34 isolates that appeared to belong to sections *Elegans* and *Martiella*, were saved for further study. Only 1 isolation of *Fusarium solani* var. *eumartii* was obtained.

In preliminary pathogenicity tests 20 to 25 plants were inoculated with each isolate by stem punctures below the ground at about the time the plants were emerging. A similar number of plants were grown on inoculated<sup>2</sup> soil previously sterilized. Not all of these inoculations were made at the same time, even with a single organism; the tests extended over a 2-year period.

Twenty-four isolates belonging in Section *Elegans* were tested in the above manner and, of these, 13 were pathogenic, producing a wilt with vascular discoloration but not resembling the symptoms of the plants from which they were isolated. In culture all of these pathogenic isolates appeared morphologically similar to *Fusarium oxysporum* and the type of wilt was similar to that produced by known cultures of that species.

Ten isolates belonging to Section *Martiella* were similarly tested and 5 of these were pathogenic. All 5 pathogenic forms appeared morphologically similar to *Fusarium solani* (Mart.) App. and Wr. One of these cultures (No. 242) produced a disease resembling that observed in the field and further tests showed conclusively that this organism was capable of causing all of the symptoms of the disease and could be isolated in pure culture from the infected tissues of inoculated plants. During 4 successive years in the greenhouse, this organism has consistently produced a high percentage of infection, as shown in table 1. Infection resulted in a much more uniform expression of symptoms than had been obtained in previous work with other species of potato *Fusaria*. In order to test relative virulence and to study resulting symptoms, one series of comparable inoculations was made with cultures of *F. oxysporum*, *F. solani* var. *eumartii*, and *F. avenaceum*.<sup>3</sup> The inoculations were made by stem punctures and by growing plants in inoculated soil previously sterilized and in similarly inoculated, unsterilized soil. The results are presented in table 1.

<sup>2</sup> The inoculum for the soil was prepared by growing the organism on sterile barley or in sterile soil to which a little bran and sugar were added. About 1 pint of inoculum was used for 25 lbs. of soil.

<sup>3</sup> The cultures of *Fusarium oxysporum* and *F. solani* var. *eumartii* were obtained from L. L. Cash of the U. S. Department of Agriculture in February 1936. These cultures had proved pathogenic in previous greenhouse experiments. *F. avenaceum* was obtained from John G. McLean, University of Wisconsin, in September 1937.

TABLE 1.—Comparative pathogenicity tests with 4 species of *Fusarium* and a summary of all inoculations with *F. solani* No. 242

Cultures	Number of plants inoculated	Number healthy	Number questionable	Number infected
Stem inoculations				
<i>F. avenaceum</i> . . .	10	6	0	4
<i>F. oxysporum</i>	10	2	0	8
<i>F. solani</i> var. <i>eumartii</i> . . .	10	0	0	10
<i>F. solani</i> No. 242	10	1	1	8
Summary of all <i>F. solani</i> No. 242 inoculations	195	38	23	134
Sterilized soil inoculated				
<i>F. avenaceum</i> . . .	29	16	7	6
<i>F. oxysporum</i>	18	9	4	5
<i>F. solani</i> var. <i>eumartii</i> . . .	20	0	0	20
<i>F. solani</i> No. 242	27	0	0	27
Summary of all <i>F. solani</i> No. 242 inoculations	101	3	13	85
Non-sterilized soil inoculated				
<i>F. avenaceum</i> . . .	24	10	5	9
<i>F. oxysporum</i>	25	9	4	11
<i>F. solani</i> var. <i>eumartii</i>	25	0	0	25
<i>F. solani</i> No. 242	25	2	3	20

There were great differences in the percentage of infection and in the type of disease produced by these different species. Inoculations with *Fusarium solani* var. *eumartii* not only resulted in 100 per cent infection but the plants grown in inoculated soil, regardless of whether or not the soil had been previously sterilized, were all dead 4 to 5 weeks after emergence, and the stem-inoculated plants showed definite symptoms in about the same length of time. The infected plants all showed a bronzing and yellowish mottling of the leaves followed by wilting. Small, brown necrotic areas were present in the pith from the base of the stem to the top leaflet. Vascular discoloration, often with an accompanying rot, was present in the underground stems. A number of the tubers showed typical stem-end rot or vascular discoloration in the stem-inoculated plants. None of the plants grown in inoculated soil produced tubers.

*Fusarium oxysporum* and *F. avenaceum* did not cause so high a percentage of infection. Neither species produced a rapid wilt, all plants remained alive for 3 months with only a slight amount of yellowing. The data in table 1 regarding these 2 species are based entirely on vascular discoloration of the stem and in a very few instances of stolons and tubers.

*Fusarium solani* No. 242 resulted in almost as high a percentage of infection as *F. solani* var. *eumartii* but wilting did not occur. With abundant soil moisture some of the plants produced aerial tubers and the leaves sometimes became tinted with purple around the margins. Rosetting of the top occurred occasionally. These secondary symptoms would probably have been more severe with higher soil moisture and lower temperatures than were

maintained in the greenhouse ( $20^{\circ}$  to  $25^{\circ}$  C.). The underground stems were always rotted, varying from a slight basal rot to a complete shattering or shredding of the stem (Fig. 1). The organism could be isolated easily from

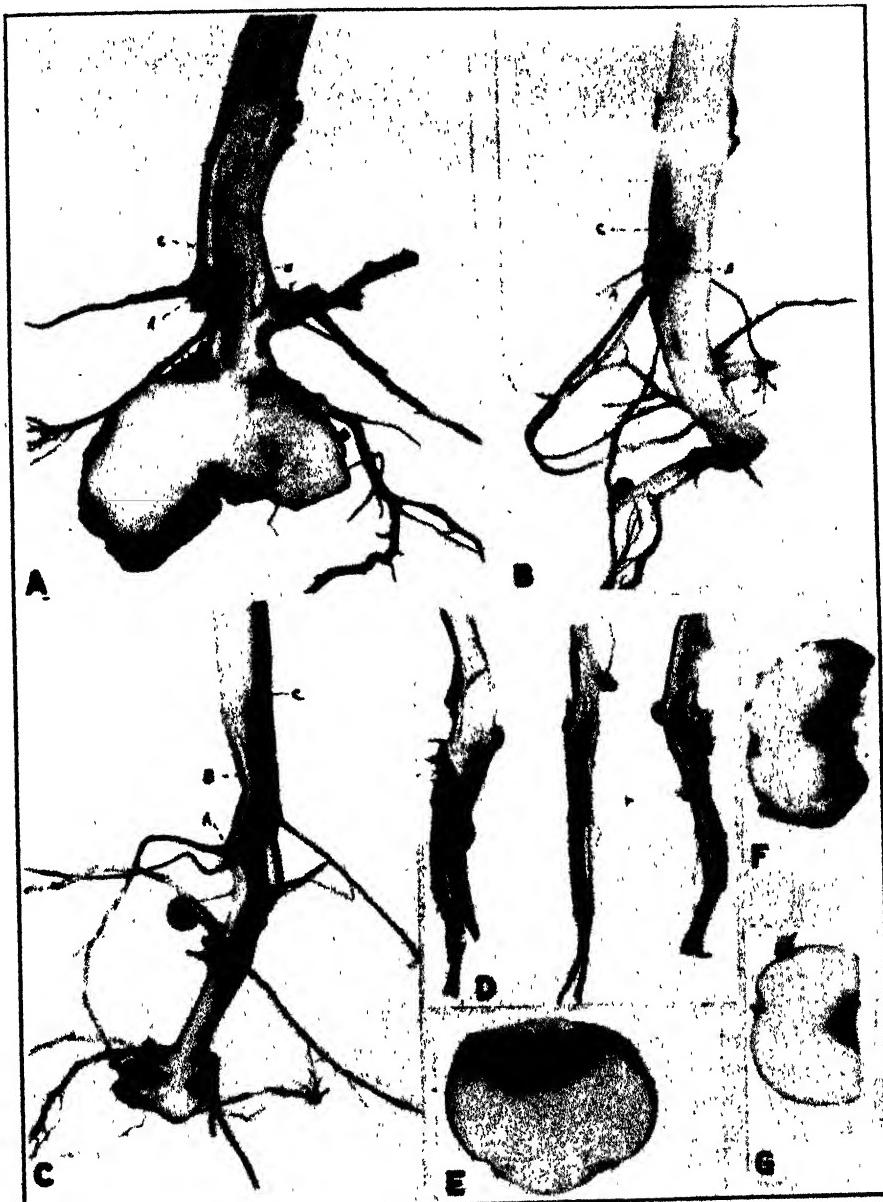


FIG. 1. A-C. Stages in the development of stem rot in Bliss Triumph plants grown in nonsterilized soil inoculated with *Fusarium solani* No. 242. Small capital letters indicate points from which the organism was isolated. D. Stems from which the original isolations were made. These are similar to the final symptoms of inoculated plants. E-F. Triumph tubers 12 and 28 days, respectively, after inoculation with *F. solani* No. 242. G. Tuber 28 days after inoculation with the squash strain of *F. solani*.

the necrotic areas of the underground stem even in the most advanced portions of the discolored pith. Evidently toxic substances causing discoloration in advance of actual penetration do not occur as with *F. solani* var. *eumartii*.

No special study was made of the mode of penetration, but the symptoms usually first appeared at the base of a root, as shown in figure 1 A, B. The absence of roots in the final stages of the disease (Fig. 1, D) indicates that this organism causes both a root and stem rot. It is possible that initial infection may occur on the roots. The emphasis placed on the stem-rot phase of the disease in this paper is due to the conspicuous and characteristic symptoms of affected stems which serve to distinguish this disease. The symptoms of the root-rot phase were indistinguishable from cortex rots of the roots due to a number of other causes.

#### IDENTIFICATION OF THE FUNGUS

A comparison of culture No. 242 with cultures of *Fusarium solani* showed it to be morphologically similar to this species.<sup>4</sup> It has not been possible to compare it with all the varieties and forms of this species other than var. *eumartii*, from which it is quite distinct.

*Fusarium solani* is often considered as a common soil fungus capable of attacking weakened plant tissues or acting as a secondary invader. It also has been reported as causing root rots of a number of unrelated species of plants and the varieties and forms all typically cause root rots. As far as the author is aware, it has not previously been reported as parasitic on roots or stems of healthy potato plants.

Inoculations were made in the greenhouse with a culture of *Fusarium solani*<sup>5</sup> isolated from squash, but the organism was not pathogenic on potato plants. Likewise, many other cultures, morphologically similar to *F. solani* and isolated from potato plants by the writer, have failed to produce the typical symptoms described in this paper; but some of them have been weakly pathogenic, causing limited cortex necrosis or slight vascular discoloration near the point of inoculation. Some variation has likewise been found in the virulence of different single-cell isolates of culture No. 242, but all of them were pathogenic and produced the same type of infection. A more detailed study of this strain in comparison with other strains of *F. solani* might reveal morphological differences not detected in this investigation.

#### TUBER ROT

The ability of this organism to cause a tuber rot as a wound parasite was tested in comparison with *Fusarium solani* var. *eumartii*. Inoculations were made by inverting a square centimeter of an agar culture on a tangential cut of a disinfected Bliss Triumph tuber and placing in a moist chamber at about 22° C. Three single-cell strains of the organism were tested, using 9

<sup>4</sup> Cultures of this organism were submitted separately to Otto Reinking, C. D. Sherba-koff, and Wm. C. Snyder, and were provisionally identified by them as *Fusarium solani*.

<sup>5</sup> This culture was kindly provided by Wm. C. Snyder of the University of California.

tubers for each strain. These tubers were cut open and examined at the end of 12 days (Fig. 1). The rotted tissue extended 10 to 15 mm. in depth and was light brown, soft, and contained cavities filled with hyphae. Some tubers showed a sharp line of demarcation between diseased and healthy tissue, while in others there was a softening of the tissue preceding the discoloration. In some, the browning extended further into the vascular tissue than into the pith. In contrast, *F. solani* var. *eumartii* produced a rot only about one-third as extensive and was darker brown and without cavities.

In another test including the strain of *Fusarium solani* from squash, the tubers were examined 25 days after inoculation. The squash strain was only slightly pathogenic on potato tubers, as shown in figure 1. The rot was confined to the tissue directly below the inoculum, whereas Strain No. 242 grew over the entire surface of the wound and penetrated all tissues below the wound.

#### TEMPERATURE RELATIONS

Preliminary tests in which the daily increase in diameter of giant Petri-dish cultures was measured indicated that the organism is favored by relatively high temperatures. The cultures were held in incubators maintained at temperatures varying from 5° to 35° at 5° C. intervals. Maximum growth occurred at 30° (84 mm. diam.) with good growth at 35° C. (34 mm.). There was only slight growth at 10° (2.5 mm.), while no growth occurred at 5° C. during the 9 days the cultures were held at these temperatures.

#### SUMMARY

A disease of potato is described that is characterized by a dry, shredded rot of the underground stem and the destruction of the roots resulting either in wilt or with high soil moistures, a rosetting and purpling of the leaves and the formation of aerial tubers.

The cause was found to be a strain of *Fusarium* morphologically similar to *Fusarium solani*. The disease was produced by inoculation of stems or by growing plants in inoculated soil either previously sterilized or unsterilized. The organism could be isolated from any of the discolored or rotted tissues.

Infection tests with this organism in comparison with *Fusarium oxy-sporum*, *F. avenaceum*, and *F. solani* var. *eumartii* showed it to be more virulent than the first two species but less so than *F. solani* var. *eumartii*. Other isolates from potato, morphologically similar to *F. solani*, and a strain from squash were not pathogenic on potato plants and only weakly so on tubers.

The organism is capable of causing a rot of potato tubers as a wound parasite, but has not been observed to infect tubers through the stolons.

It was found to be favored in pure culture by high temperatures (optimum 30° C.).

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## FLOWER BLIGHT OF CAMELLIAS

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(Accepted for publication August 18, 1939)

The flower blight of Camellias (*Camellia japonica* L.), caused by a species of *Sclerotina*, was first found in February, 1938, near Hayward, California, in a nursery where Camellias are grown under lath and in the open for cut flowers and for garden plants. Apothecia were found in abundance under the plants grown in the lath house, whereas neither apothecia nor sclerotia were found under those grown in the open where field cultivation was practiced and no mulches used. Apparently, the flowers grown in the open became infected by wind-borne spores produced in the adjacent lath house.

Only the floral parts are affected, and all of the more than 50 varieties of Camellias grown in this nursery appeared to be equally susceptible. The disease is highly specific to this one host, as shown by the fact that no infections were found on flowers of Rhododendrons, Azaleas, Magnolias, Gardenias, Paeonies and many other flowering shrubs, though they were grown under the same lath, intermingled with Camellias. We have examined several places in the State where Camellias are grown and made inquiries at others, but obtained no evidence that the disease had been observed in any of them. The only suggestion of a possible source of this new disease may be seen in the fact that Camellias are native to the Orient and are still being freely imported from there.

The occurrence of the disease coincides with the flowering period of Camellias, roughly from February to May, which is also the season of rather frequent rains. During that period the losses vary from a relatively small percentage in dry weather to 100 per cent of all open flowers for several days following rains. Under such humid conditions it is unsafe to cut and ship even the flowers that appear to be unaffected, as they become badly spotted in transit and storage.

### SYMPTOMS

Infection of the individual flower may take place soon after the tips of petals are visible in the opening bud or at any time thereafter. Few to many small, irregular, brownish specks appear on the petals of expanding flowers (Fig. 1, A, B). Under favorable conditions of temperature and moisture these specks rapidly enlarge and unite to form large spots (Fig. 1, B) which soon involve the entire petal and eventually the whole flower, which becomes uniformly dull brown and drops from the plant. Even one or two infections may suffice to render the flower unsalable. When infection begins near the base of the petals the entire center of the flower may be killed, while the tips of the petals retain their normal form and color. There is no rapid disintegration of invaded tissues, hence infected flowers retain their shape and firmness for many days after they have turned completely brown and fallen

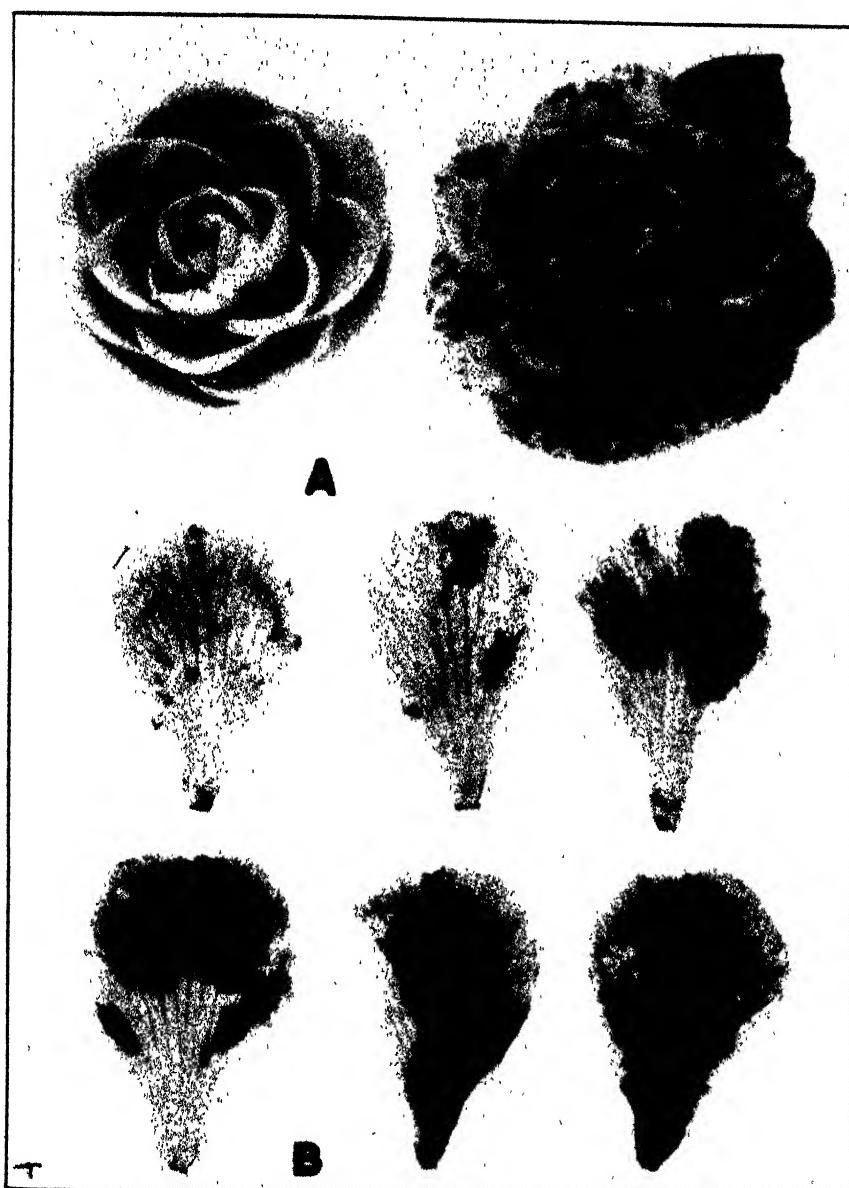


FIG. 1. A. Camellia flower 24 hours after being inoculated with ascospores of *Sclerotinia camelliae* (control on left). B. Individual petals from naturally infected flowers showing early and late stages of the disease.

to the ground. While the flowers are resting on the wet earth, microconidia are often produced on the petals in shiny, black streaks or masses, giving the flowers the appearance of being affected with a wet rot. After the flower is completely blighted the causal fungus continues to develop within the basal parts of the petals, but soon grows beyond the petal limits (Fig. 2, D) to form hard, dark-brown to black sclerotia that frequently unite at the base

to form a laminated compound structure simulating the imbricate petal arrangement in the flower (Fig. 2, A).

#### LIFE HISTORY OF THE FUNGUS

The sclerotia lie dormant on the ground or covered with soil or mulching materials under the bushes during the summer and early part of the winter. As the period of Camellia bloom in early spring approaches, some of the sclerotia become active, while others remain dormant for another year or possibly longer. After a period of wet weather with rising temperature, they then begin growth and produce from one to several apothecia each. Apothecial formation is apparently greatly stimulated by spring applications to the soil of top-dressing materials, such as barnyard manure, peat, etc. In cases where the sclerotia are near the surface, the apothecia may be nearly sessile or, where deeply buried, the stipes may be up to 40 mm. in length (Fig. 2, B). The saucer-like discs of the apothecia vary from 5 to 20 mm. in diameter. The ascospores are discharged forcibly into the air and are carried by wind currents to the flowers, where they germinate, invade the petal tissues, and eventually produce sclerotia and microconidia, thus completing the life cycle. Flowers at the top of a 15-ft. bush appear to become infected as readily as those produced near the ground. The microconidia have not been observed to germinate and no secondary conidia are formed.

#### BEHAVIOR ON ARTIFICIAL MEDIA

On potato dextrose agar the fungus first forms a very close, felty, cream colored mat which begins to turn darker in about 10 days and becomes jet-black and shiny in about 25 days, the change in color being due to the presence of microconidia, which are produced in abundance over the entire mat. Occasionally small, flat, black sclerotia are formed that rarely attain a size of more than  $2 \times 5$  mm. On sterilized whole wheat, well-rounded sclerotia up to 7 mm. in diameter have been produced. The fungus produces microspores sooner and more abundantly at room temperature, about  $24^{\circ}$  C., but mycelial growth and sclerotium production is much more rapid at 15 to  $18^{\circ}$  C.

#### INOCULATION

Six detached healthy Camellia flowers were sprayed with water and suspended for 1 hour over mature, spore-discharging apothecia. Six others were sprayed with a water suspension of ascospores. After inoculation the flowers, together with noninoculated controls, were placed in moist chambers. All the inoculated flowers showed typical spotting after 24 hours (Fig. 1, A) and became completely browned in 48 hours, with sclerotial formation well started. The controls remained unspotted.

A careful search of the literature failed to reveal any Discomycete associated with Camellias or with other members of the family *Theaceae*. In view of this, and on the basis of certain distinct morphological features, to-



FIG. 2. A. Typical petaloid sclerotia. B. Short-stipe and long-stipe apothecia. C. Part of cross section of apothecium.  $\times 380$ . D. Part of cross section of sclerotium.  $\times 185$ .

gether with the host specificity of the pathogen, we conclude that it is new to science and propose for it the name *Sclerotinia camelliae*, sp. nov.

#### TECHNICAL DESCRIPTION OF THE PATHOGEN

*Sclerotinia camelliae*, sp. nov. Apothecia singly or in groups, buff-olive becoming darker with age, scantly pubescent; disc cyathiform becoming discoid, 5–20 mm. in diameter; stipe 3 to 40 mm. long, 2–3 mm. in diameter below disc tapering to 0.5–1.0 mm. at base. Ascii cylindrical 4.3–5.8 × 100–125  $\mu$ . Ascospores 8, uniseriate, ellipsoid, continuous, hyaline 2.5–3.5 × 5.3–7.0  $\mu$ . Paraphyses filiform, septate 1.2–2.5 × 110–130  $\mu$ , tips slightly swollen. Sclerotia dark-brown to black, usually compound, impregnating and surrounding the petal tissues; very variable in outline, up to 12 × 30 mm. in size; usually laminated to simulate the imbricate petal arrangement of flowers. Conidia none. Microconidia globose to pyriform 2.5–3.5  $\mu$ , catenate, hyaline under high magnification, jet-black in mass, produced in a sporodochium made up of numerous clusters of conidiophores that end in tapering elongate terminal cells on which the long chains of microconidia are produced.

#### CONTROL

Our observation during the past 2 seasons that no apothecia were found under plants grown without mulching in the open would suggest that there is little danger of the disease becoming established in parks or private gardens. In view of the facts that ascospores alone are able to cause infection and that sclerotia are formed in the flowers only, it would seem a relatively simple matter to control the disease and eradicate the pathogen by gathering and destroying all fallen flowers. This has been undertaken by the nursery involved. This would have to be done for at least 2 consecutive seasons, since it is known that the sclerotia may remain alive in the soil for at least 2 years and probably longer.

#### SUMMARY

A new disease affecting the flowers of *Camellia japonica* L. is described. Early symptoms on the petals appear shortly after late winter and spring rains as small brown specks, which soon enlarge, coalesce, and cause the whole flower to turn brown and fall. Sclerotia, formed within the flower, rest on or in the ground for one or more years after which they produce apothecia. The ascospores are wind-borne, and there are no viable conidia. The pathogen, described as new, is named *Sclerotinia camelliae*, sp. nov. Destruction of all fallen flowers for several consecutive seasons is suggested for control.

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# SOME EFFECTS OF STRAINS OF CUCUMBER VIRUS I IN LILY AND TULIP

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(Accepted for publication August 8, 1939)

On December 20, 1938, healthy seedlings of the Easter lily (*Lilium longiflorum* Thunb.) were inoculated with 5 strains of Cucumber Virus 1, including one isolated from Easter lily, each taken from Turkish tobacco, and a lily virus of the tulip group taken from Easter lily seedlings. After 34 days all plants inoculated with the cucumber-virus strains remained essentially symptomless, but those inoculated with the tulip virus expressed characteristic strong mottling from about 14 days onward (Fig. 1, A). On subinoculation from each set of 5 lily plants to Turkish tobacco, all 5 cucumber strains were recovered (Fig. 1, B) but the tulip virus was not. In other trials the common lily strain of cucumber mosaic has been passed through both seedling Easter lilies and through *L. formosanum* Stapf. many times, and through *L. speciosum* Thunb. once, without inducing well-defined symptoms. From about May 15 onward, when the greenhouse temperatures rise above control in this latitude, a mild yellow mottling has appeared in young leaves of *L. formosanum* inoculated with lily strains of cucumber mosaic some days or months previously. No well-defined effects in inoculated Easter lily seedlings or in *L. speciosum* accompanied this seasonal rise in temperature.

Flecking, roughly comparable to the necrotic-fleck type seen in commercial Easter lilies, has been produced by inoculating a lily strain of cucumber mosaic into commercial Easter lilies, shown by previous subinoculation to carry McWhorter's (3) "latent virus of lily" (Fig. 1, C). The most plausible explanation of the discrepancy between our results and Price's (5) report of fleaek symptoms induced by cucumber-virus strains alone is that some of Price's "selected cage-grown stock" carried the latent virus reported by McWhorter, i.e., a virus of the tulip group, typically latent in *Lilium longiflorum*. Furthermore, Wellman's (6) gray mottle and flecking in Easter lilies inoculated with the celery strain indicates that the inoculated Florida stocks already carried a virus of the tulip group.

Cross inoculations of viruses from tulip to cucumber and tobacco, and of the cucumber-virus strains from cucumber to tulip were negative in our trials until this year (2). However, on March 30 and May 2, 1939, we twice successfully subinoculated to tobacco the celery strain and a lily strain of Cucumber Virus 1 from Clara Butt tulips inoculated with these viruses on April 16, 1938. Parallel subinoculations to tobacco from Clara Butt tulips inoculated with viruses of the tulip group from tulip and lily were negative. Type material of McWhorter's (4) tulip viruses 1 and 2 readily produced symptoms in *Lilium formosanum*, but no infection in Turkish tobacco.

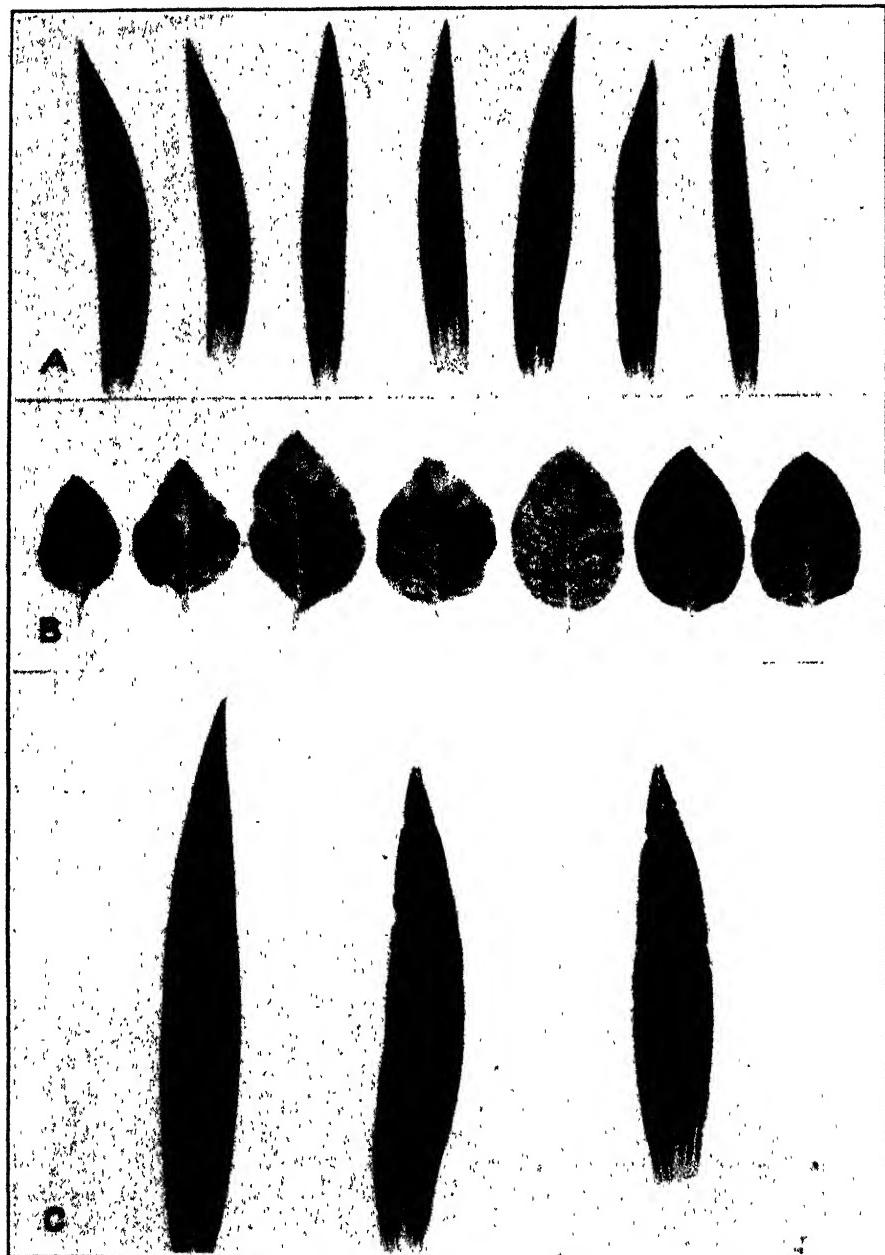


FIG. 1. A. Representative leaves from each of seven sets of Easter lily seedlings inoculated with (left to right) Doolittle's strain, a second isolate similar to the first, Price's strain, celery strain, and a lily strain of Cucumber Virus 1, the strong mottle virus (tulip type) from Easter lily, and uninoculated control. B. Representative leaves from sub-inoculations to Turkish tobacco from the corresponding Easter lily inoculations shown above. C. Representative leaves from three sets of an Easter lily clon carrying McWhorter's latent virus of lily (tulip virus). Left to right: uninoculated control, symptomless; fleck symptoms following inoculation with a lily strain of Cucumber Virus 1; fleck symptoms following inoculation from typical flecked Easter lily.

Clara Butt tulips, from which the celery and lily strains of cucumber virus were reisolated, showed longitudinal gray streaks in the leaves at about the time the blooms began to show color. The flowers were broken (Fig. 2, B) with a dull break, the margins of the stripes being less sharply defined than in tulip mosaic (Fig. 2, C). The outer perianth parts usually showed



FIG. 2. Two types of flower breaks in Clara Butt tulips. Left to right: A. Healthy. B. Broken from inoculation April 16, 1938, with the common lily strain of Cucumber Virus 1. C. Natural infection with tulip mosaic.

a characteristic blemish (Fig. 2, B) and were shorter than normal. The blemish was gray at the base, sometimes greenish at the summit. Subinoculation from plants showing this atypical break to Turkish tobacco were uniformly successful in recovering the celery or the introduced lily strain, while parallel subinoculations to *Lilium formosanum* gave no evidence of the presence of tulip virus. The symptoms illustrated are, therefore, those of cucumber virus (lily strain) in tulip. The type has not been observed by the writers in previous experience with tulips.

The susceptibility of tulips to other strains of Cucumber Virus 1 was expected after Ainsworth (1) reported isolation of a strain of this group from tulip. Our results show that a cucumber virus (celery strain or lily strain) may be introduced into tulips and induce breaks in the blooms, but that cucumber strains are not associated with the usual breaking of tulips. In Easter lilies, strains of cucumber mosaic are recoverable from plants thus far sampled showing any of the distinctly injurious symptom types, but the cucumber strains alone are, with one known exception, not markedly damaging to Easter lilies. It is increasingly evident that lilies are common hosts for members of the tulip and the cucumber 1 groups of viruses, but there is no evidence from our work, or from any yet published by others, to indicate that these groups are closely allied.

We have not found cucumber virus strains occurring naturally in tulips nor yet alone in commercial Easter lilies. In our tests, when such strains are experimentally introduced into seedling Easter lilies, they have not induced

symptoms with the exception of one virulent strain, associated with yellow top symptoms rather than fleck symptoms. McWhorter's (3) "latent virus of lily" is very commonly present in symptomless Easter lilies, excepting suitably protected seedlings. It is reasonable to assume that the tulip type latent is so prevalent in commercial Easter lily stocks that infection with a cucumber strain commonly produces a complex with recognizable symptoms.

It is of interest that cucumber mosaic strains, experimentally introduced into tulips, produced no recognized effects in the current season, but induced flower breaks in the following year, as do the tulip viruses. On the other hand, the tulip viruses from lily and tulip, including those received from McWhorter as type material, induce symptoms in *Lilium formosanum* in 2 weeks. The long incubation period of the classical tulip breaking is, therefore, evidently a peculiarity of the tulip rather than of the viruses involved.

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#### DEVELOPMENT OF SCAB ON STORED APPLES, 1938-1939

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(Accepted for publication September 21, 1939)

In February, 1939, the writer observed several lots of apples from Pennsylvania, New Jersey, Massachusetts, and the Hudson Valley of New York on the New York City market bearing many small, black, scab lesions typical of those that develop in storage. The lesions on Stayman Winesap, Baldwin, and Stark varieties were mostly from  $\frac{1}{8}$  to  $\frac{1}{4}$  inch in diameter and were jet black. Lesions on Rome Beauty apples were smaller, being generally less than  $\frac{1}{16}$  inch in diameter, and were dark brown. A few of the lesions, particularly those on riper fruits, were larger, caused no roughening of the cuticle of the apple, and resembled "ink spots." The cuticle on most of the lesions, however, was roughened and bore low fungus growth. Typical lesions, as they appeared on apples of these 4 varieties, are shown in figure 1. The Stayman Winesap apple in the photograph bore a total of 553 lesions.

Some of the scabbed lots were traced to their points of origin in the eastern part of Pennsylvania, where the writer examined additional lots of apples in storage and interviewed growers and cold-storage warehouse managers concerning treatment of the fruit. Many of the lots of Stayman Winesap and Rome Beauty showed from 80 to 90 per cent of the fruits in-

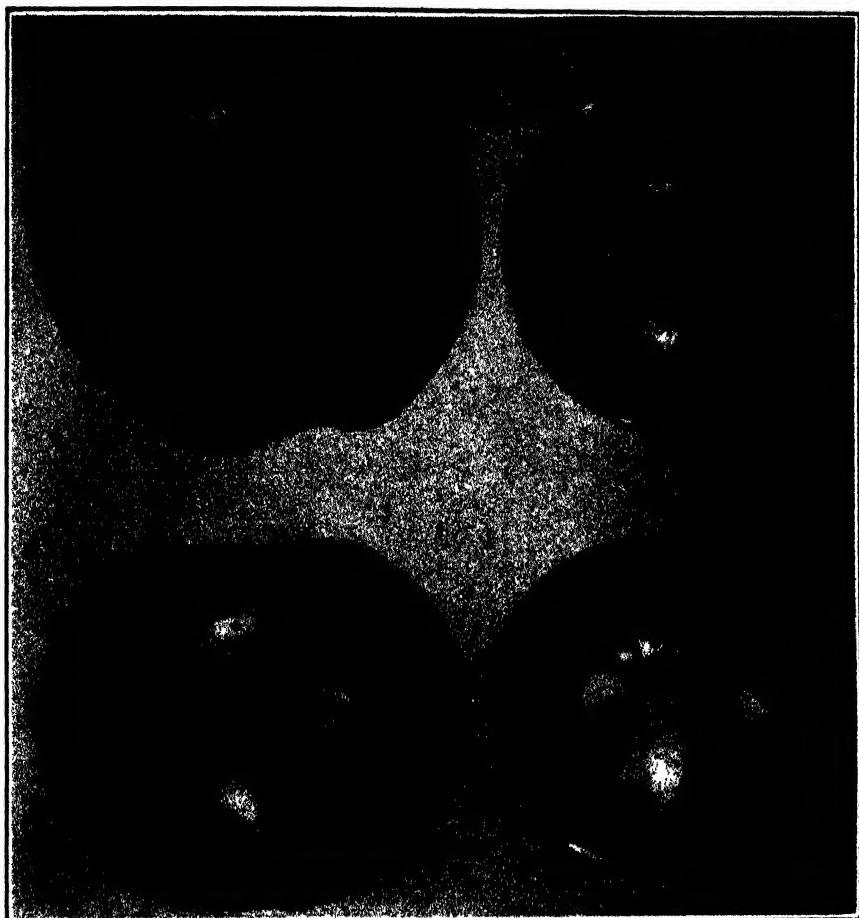


FIG. 1. Scab on apples held in cold storage for 4 months. Apples on left from Pennsylvania, Stayman Winesap above, Rome Beauty below; apples on right from Hudson Valley of New York, Baldwin above, Stark below.

fected. Apples from 1 orchard stored in 3 different cold-storage warehouses were equally affected, indicating that any slight differences in storage conditions that the fruit may have been subjected to had little effect on scab development. McIntosh and Delicious apples, picked from the same orchard and stored in the same cold storage as that of the heavily infected fruits of Rome Beauty and Stayman Winesap varieties, were found to have remained scab-free. By reference to the records kept by the grower, it was learned that the first named varieties were picked and stored during the first 3 weeks of September, whereas the Rome Beauty and Stayman Winesap varieties were picked during the first 3 weeks of October.

The growers' records showed that the orchards from which the scabbed Pennsylvania apples came received the final spray application during the first week of July. Foliage scab was apparently so well controlled that a later spray was not considered necessary. It was stated that at picking

time foliage scab was slight and very few fruits bore visible scab lesions. No new lesions were seen on the stored fruit by those who inspected it until January, when very small spots were seen on an occasional fruit.

Information concerning the prevalence of scab on stored fruit grown in other sections of the Northeast was obtained through correspondence with growers and inspectors of the Agricultural Marketing Service of the U. S. Department of Agriculture. The McIntosh variety in the Hudson Valley, which was picked mostly during the first 3 weeks of September, remained nearly scab-free, whereas the Baldwin, Stark, and Rome Beauty varieties, picked in October, became in many cases severely spotted during storage. Reports from New Jersey and New England indicate that only those varieties picked in October became heavily infected. Orchardists in Connecticut stated that the windfalls picked up and stored immediately after the hurricane of September, though not held so long as the fruit picked afterward, showed little scab development, whereas fruit picked later showed heavy infection. Growers in Massachusetts reported that heavy storage infection occurred on Baldwin and other varieties of apples picked in October. Contrary to the general situation throughout the Northeast, little scab development on stored apples was reported from western New York.

Inoculation experiments reported earlier by the writer<sup>1</sup> showed that apple fruits could be successfully inoculated at any stage of their development on the tree provided they were subjected to increasingly longer periods of wetness as they enlarged. Apples nearing maturity and mature fruits picked from the tree were successfully inoculated when kept moist at orchard temperature for 7 days. If the apples were picked and stored immediately after the 7-day period, the resulting infection did not appear until the normal storage life of the fruit was passed; but, if they were allowed to remain on the tree for a week or longer, the latent period of infection was shortened to from 2 to 4 months.

In an attempt to correlate this outbreak of scab with weather conditions in late summer, weather reports were obtained from U. S. Weather Bureau Stations in representative cities in the Northeast. Throughout this section rains occurred on several successive days in early August but, at most stations, high percentages of sunshine were recorded on the days of the rains. Foliage and fruit apparently did not remain wet long enough during this period to allow serious infections, for resulting lesions should have appeared on the fruit by late September or early October.

Only light rains on occasional days were reported at any of the stations during the early part of September. But starting September 12 and lasting through the hurricane, 9 days later, light to heavy rains occurred generally throughout the East. At New York City some rain fell daily during that period, and at other nearby stations only 1 or 2 days were entirely rain-free. Table 1 shows daily measure of precipitation at the 7 stations from which records were obtained. With the exception of those for Rochester, N. Y.,

<sup>1</sup> Bratley, C. O. Incidence and development of apple scab on fruit during late summer and while in storage. U. S. Dept. Agr. Tech. Bull. 563. 45 p. 1937.

TABLE 1.—*Precipitation recorded from September 11 to 22, 1938, by the U. S. Weather Bureau at 7 stations in the Northeast*

Station	Precipitation in inches per day in September											
	11	12	13	14	15	16	17	18	19	20	21	22
Reading, Pa.	T	T	.05	.23	.01	0	.31	.01	.60	1.77	1.12	0
Trenton, N. J.	0	0	.02	T	.54	T	.34	.38	2.46	2.27	2.67	0
New York, N. Y.	0	T	.13	T	.66	T	.12	.39	1.80	1.73	3.71	0
Albany, N. Y.	0	.45	.24	.23	.26	0	.25	.01	1.78	1.25	3.25	0
Rochester, N. Y.	0	.26	.04	.03	.08	T	0	.17	0	.65	1.33	0
Hartford, Conn.	0	.07	.27	T	.51	0	.60	1.45	1.80	6.10	3.22	0
Boston, Mass.	0	.27	.65	0	.36	0	.56	1.19	.67	1.50	.10	0

the records show moderate rainfall from September 12 to 15, then one day, the 16th, with little or no rain followed by very heavy rains daily from the 17th to and including the 21st. During the latter period of 5 days little or no sunshine was reported and relative humidity averaged 90 per cent or above most of the time. These 5 days, preceded as they were by an almost equal period of intermittent rains, undoubtedly afforded optimum conditions for inoculation of apple fruits. This is borne out by the fact noted above that only those apples picked after September 21 showed extensive development of scab in storage.

As may be seen by reference to table 1, Rochester, N. Y., did not receive the heavy, almost continuous rains from September 17 to 21. There was some rain daily at this station during the period from September 12 to 17, but with the exception of the 12th, 14th, and 15th, the daily percentages of sunshine reported were quite high and the percentages of relative humidity fairly low. It is, therefore, interesting to note that no more than the usual amount of scab development in storage was reported on fruit from western New York of which Rochester is the center.

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## THE SNOW MOLDS OF GRAINS AND GRASSES CAUSED BY TYPHULA ITOANA AND TYPHULA IDAHOENSIS

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### INTRODUCTION

A great deal of confusion exists in phytopathological literature concerning the identity of the organisms causing the snow molds of grains and grasses. The sclerotial fungi that have been found associated with these diseases have been referred at various times to *Sclerotium rhizodes* Auerswald<sup>1</sup> (2), *Scl. fulvum* Fries (11), *Typhula graminum* Karst. (9, 10) and *T. itoana* Imai (4). This confusion has come about partly because of the macroscopic resemblance of the sclerotia of the above-named fungi as described by early mycologists, and partly because of the inability to collect or otherwise obtain fertile sporophores, which are essential for taxonomic classification. A recent study made by the writer (8) has cleared up this situation, and the species of *Typhula* associated with these diseases have been identified.

### REVIEW OF LITERATURE

The snow molds have been of considerable economic importance in the United States, as well as in Europe and Japan. They are associated with cold weather and snow, and are most frequently found where the snow is deep or drifted and, consequently, delayed in melting in the spring. When

<sup>1</sup> According to H. H. Whetzel, *Sclerotium rhizodes* Auers. is a *Ehzoctonia*. Unpublished data.

the snow melts, the plants are found covered with a moldy, white fungous growth, and the tissue is filled with small reddish brown or dark brown sclerotia. These diseases have caused considerable damage in Idaho (7) and Montana (11), and *Typhula itoana* is becoming of increasing importance on turf and lawn grasses in the eastern United States. For several years *T. itoana* has been the cause for much concern in Japan (5, 9) and frequently has been reported from northern Europe (6, 10) and the Scandinavian countries (1), usually under the name *T. graminum* Karst.

#### TAXONOMIC POSITION OF THE FUNGI

Since it is possible to fruit these fungi abundantly and at will in either diffuse natural daylight or under ultra-violet irradiation (8), it is an easy matter to identify them. The fungus most commonly found is *Typhula itoana* (3), which produces tawny to hazel-brown sclerotia from which develop rose-colored sporophores, 8–25 mm. tall. An examination and comparison of cultures obtained in the United States with those from Japan<sup>2</sup> and Europe<sup>3</sup> show that the same organism, *T. itoana*, is the cause of a snow mold in these three countries. Ekstrand (1) has called attention also to the fact that the most common species causing this same disease in Sweden is *T. itoana*.

A second species of *Typhula* has been found associated with snow mold in Idaho and Montana. This one produces small chestnut brown sclerotia from which develop fawn to wood-brown sporophores, 5–10 mm. tall. This species has been described as *Typhula idahoensis* (8), and causes a disease of the same type as that caused by *T. itoana*.

Ekstrand (1) mentions in addition to *Typhula itoana*, a small brownish black sclerotium, which is frequently isolated from diseased cereals and grasses in northern Sweden. He has given the name *T. borealis* to the sclerotial stage of the fungus, but does not describe the sporophores. From his short description of the sclerotia it seems possible that he is dealing with a species similar to, if not identical with, *T. idahoensis*.

It is necessary to study the sclerotial morphology of species of *Typhula* before a proper identification can be made. Sclerotia of different species often resemble each other very closely macroscopically, but have an entirely different arrangement of rind and medulla, and differ also in the characters of the sporophores they produce. The morphological construction of sclerotia of *T. itoana* is identical with that of *Sclerotium fulvum* Fries<sup>4</sup>; that is, the rind is a homogeneous gelatinous layer and the medulla paraplectenchy-matous (8). It is safe to assume that these two organisms are the same. On the other hand, a critical examination of the type specimen of *T. graminum* Karst.<sup>5</sup> shows the sclerotia to be of a different morphological type.

<sup>2</sup> Obtained through the courtesy of H. Tasugi, Tokyo, Japan.

<sup>3</sup> Obtained through the courtesy of A. Volk, Königsberg, Germany.

<sup>4</sup> Roumeguère, *Fungi gallici exsiccati*, 1400, *Sclerotium fulvum* Fr., in the Herbarium, Dept. of Plant Pathology, Cornell University, Ithaca, N. Y.

<sup>5</sup> Loaned for examination through the courtesy of Dr. Harald Lindberg, Helsinki, Finland.

The medulla is prosoplectenchymatous with a layer of enlarged thin-walled cells adjacent to the homogeneous gelatinous rind. The macroscopic similarity of sclerotia of *Scl. fulvum*, *T. graminum* and *T. itoana* has doubtless led many investigators to assign all the organisms causing the snow molds to *T. graminum*. *T. graminum*, however, produces white sporophores, while *T. itoana* produces rose-colored ones. There is a possibility that *T. graminum* also causes a snow mold, but its isolation and pathogenicity have never been conclusively demonstrated by any investigator. It is interesting to note that the sporophores of *T. graminum* Karst. do not arise from the sclerotia of *Scl. fulvum* Fries, as Karsten originally assumed.

#### SUMMARY

Two species of *Typhula* are definitely associated with snow molds of cereals and grasses. The one most commonly found in the United States, Europe and Japan is *T. itoana* Imai, frequently described under the name of *T. graminum* Karsten or *Sclerotium fulvum* Fries. The second species, often collected in western United States, is *T. idahoensis* Remsberg, and is probably the organism described by Ekstrand as *T. borealis* in Sweden. There is a possibility that a third species, *T. graminum* Karsten, may be associated with the snow molds, but it has not yet been demonstrated.

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## PHYTOPATHOLOGICAL NOTES

*Potato Seed-piece Rot Caused by Fusarium oxysporum.*—In 1934, a potato seed-piece rot was observed at Hastings, Florida, in many fields within 2 to 3 weeks after potatoes were planted. The disease caused stand reductions varying from 10 to 70 per cent in 400 acres, planted in December, 1933 (Fig. 1, A). It has reappeared every year since 1934, but has caused little trouble, except in 1936, when it destroyed 10 per cent or more of the seed in most of the potato fields in the Hastings section.

Gratz<sup>1</sup> reported a *Fusarium* seed-piece rot at Hastings, Fla., in 1924 and 1925, but he did not identify the species of *Fusarium* concerned. In 1919, MacMillan<sup>2</sup> reported that infection of potatoes by *Fusarium oxysporum* Schlecht from the soil through the seed piece occurred in irrigated land in potato-growing sections of Colorado. MacMillan described the malady in detail and suggested that its severity in some soils was due to growing successive crops of potatoes on the land and to the presence of soil temperatures especially favorable for the disease.

The *Fusarium* causing seed-piece rot can be seen on the cut surface and occasionally on the cortex of decaying seed pieces 2 to 3 weeks after planting (Fig. 1, C and D). The rot starts at the cut surface, and the tissues invaded become brown (Fig. 1, B) but remain firm until secondary decay organisms enter, after which the pieces become soft and mushy. If decay is rapid, the seed pieces either fail to germinate or produce weak sprouts that may rot off before they can emerge from the soil. If the affected seed pieces decay slowly, they may produce plants bearing marketable tubers or plants that produce only a few small tubers or none when the fungus destroys the seed, invades the stems and roots, and finally causes the plant to wilt and die prematurely. Tubers of plants with affected seed pieces do not rot in the field.

Hard potato-dextrose agar of pH 5.6 was colored vinaceous purple by the fungus isolated from decaying seed pieces. The macroconidia were typically 3-septate,  $4.5 \mu \times 40.3 \mu$ , borne on branched conidiophores. Macroconidia formed in sporodochia and chlamydospores developed in aged cultures. The characteristics of the organism (Fig. 2) agree with those given by Sherbakoff<sup>3</sup> for *Fusarium oxysporum*.

In fields where the disease was severe in 1934, it was noted that seed became infected and rotted in one row where no fertilizer had been used, in rows fertilized 2 weeks prior to planting, and in rows where the seed and fertilizer had been placed in the soil at one operation.

Numerous inoculation experiments proved that *Fusarium oxysporum* isolated from decaying seed pieces was pathogenic. In one series of experiments, pure cultures of the fungus growing on small squares of potato-dex-

<sup>1</sup> Gratz, L. O. Irish potato disease investigations, 1924-25. Fla. Agr. Exp. Stat. Bull. 176. 1925.

<sup>2</sup> MacMillan, H. G. Fusarium blight of potatoes under irrigation. Jour. Agr. Res. [U. S.] 16: 279-303. 1919.

<sup>3</sup> Sherbakoff, C. D. Fusaria of potatoes. Cornell Agr. Exp. Stat. Mem. 6. 1915.

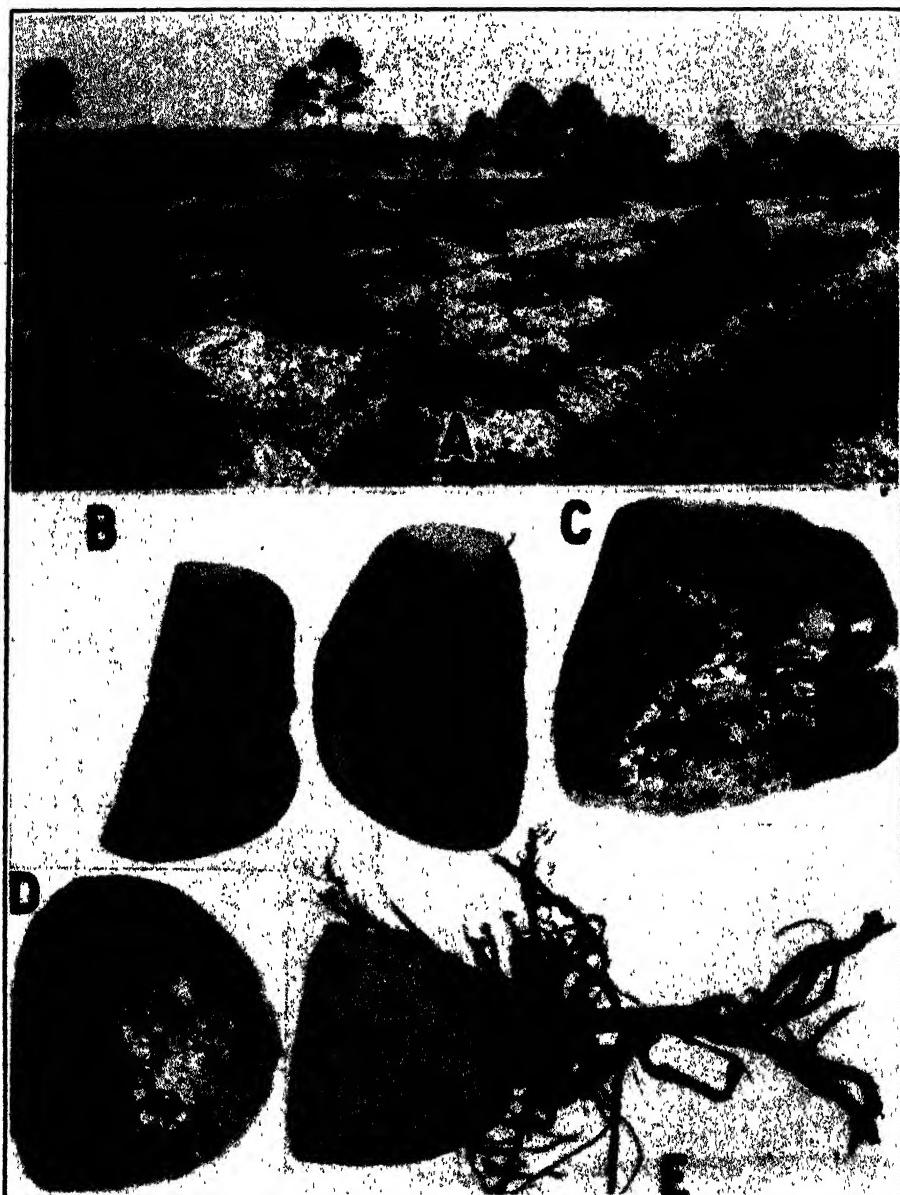


FIG. 1. *Fusarium* seed-piece rot of potato. A. Section of a field in which 65 per cent of the seed pieces were destroyed by *F. oxysporum* prior to germination. B-D. Naturally infected seed pieces: Seed piece, which did not germinate, sectioned to show discoloration caused by *F. oxysporum* (B); *F. oxysporum* growing on cut surfaces (C) and on cortex (D). E. Section of sprouted healthy seed piece.

trose agar were placed on the cut surfaces of seed pieces which had been cut one day previously and those that had been cut only a few minutes before being inoculated. The inoculated pieces were planted in sterilized soil, together with noninoculated pieces from the same seed tubers. When re-

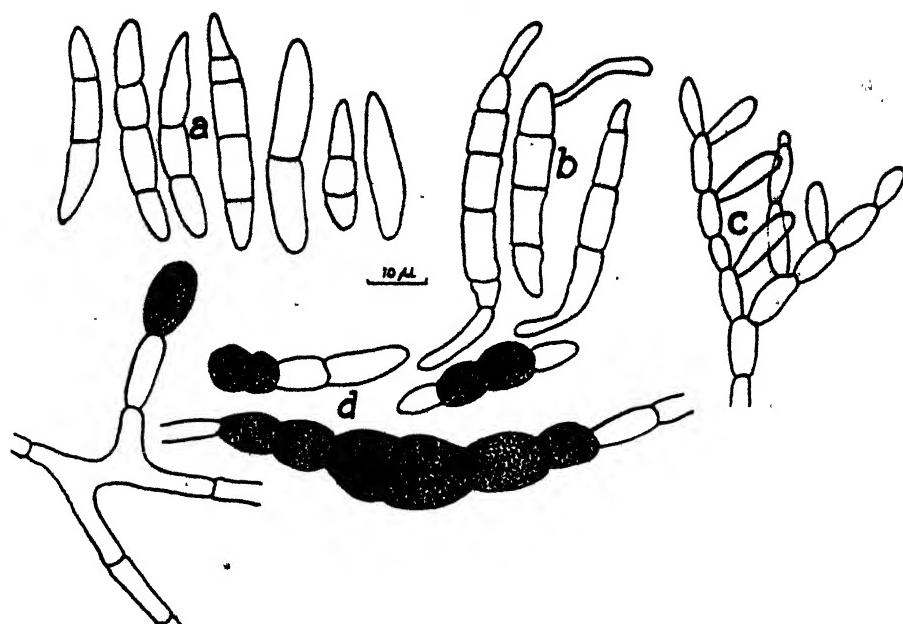


FIG. 2. Camera lucida drawings of *Fusarium oxysporum* showing (a) types of macroconidia, (b) germinating macroconidia, (c) conidiophore and (d) chlamydospores in mycelium and macroconidia.

moved from the soil 28 days later, all inoculated pieces were partly or totally decayed and either had not germinated or had produced only weak sprouts, while the pieces not inoculated were sound and had produced strong sprouts.

In another series of experiments, 25 inoculated and 25 noninoculated seed pieces from the same seed tubers were planted in a field in rows in which one ton of fertilizer per acre had been distributed 2 weeks before planting. When examined 28 days later, all inoculated pieces were partly or totally decayed and 14 of the noninoculated pieces were also rotting. *Fusarium oxysporum* was isolated from the inoculated and noninoculated pieces that were rotting.—A. H. EDDINS, Hastings Laboratory, Florida Agricultural Experiment Station, Hastings, Florida.

***Lightning Injury of Black Locust Seedlings.***—An occurrence of lightning injury of first-year black locust (*Robinia pseudoacacia L.*) seedlings, which had just emerged, was observed during July, 1936, in a forest nursery near New Brunswick, New Jersey. According to information from the nursery personnel, the lightning entered the field of seedlings from a power-line tower. The damaged spot was approximately 50 by 100 feet, and it extended from one side of the tower (Fig. 1, A). Most of the plants in the central part of the spot were killed immediately. Those in the outer part were severely injured but not killed, as shown by the various degrees of the dwarfing that appeared later.

An examination of a large number of injured plants showed that the deeper roots had been killed (Fig. 1, B). Since the roots were very short

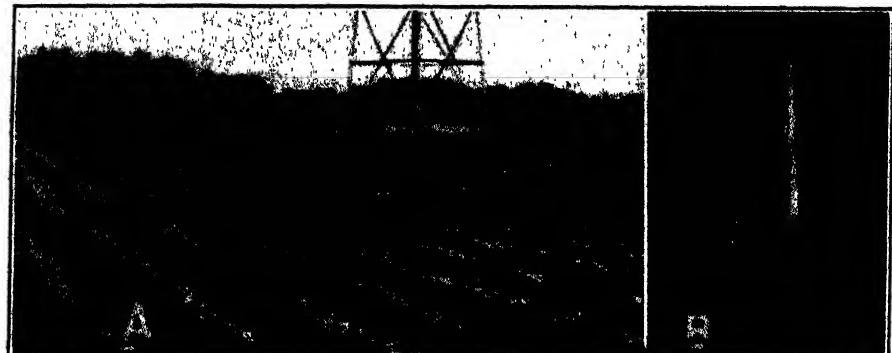


FIG. 1. Lightning injury of black locust seedlings. A. A general view of the damaged spot that extended from one side of the power-line tower. B. External view of an injured seedling showing the killed roots and the root growth that was stimulated after the injury.

and still succulent when the injury occurred, the killed tissues simply collapsed and did not show any marked internal symptoms. Jones and Gilbert<sup>1</sup> reported a case of lightning injury of tobacco where the roots were killed and had a charred appearance. There were no external symptoms of injury on the stems or leaves of the locust seedlings. Isolations from the injured roots did not yield any of the known parasitic fungi. Root growth had been stimulated above the point of injury on many of the survivors (Fig. 1, B) that were examined about 2 weeks later. Walker<sup>2</sup> reported that adventitious roots were stimulated above the point of injury on the stems of cabbage that had been injured by lightning. Most of the severely injured plants stayed dwarfed and more or less chlorotic, and either died ultimately or were considered unusable for planting purposes. This case of lightning injury is of particular interest because it may explain some of the sudden occurrences of seedling losses in fairly large spots in forest nurseries.—L. W. R. JACKSON, Division of Forest Pathology, Bureau of Plant Industry in cooperation with the Allegheny Forest Experiment Station and the University of Pennsylvania, Philadelphia, Pa.

*An Attempt to Propagate Tobacco-mosaic Virus 1 in the Chorio-allantoic Membrane of the Developing Chick Embryo.*—The chorio-allantoic membrane of the developing chick embryo has been found unusually favorable for the propagation of many different viruses affecting animal hosts. Even some viruses not infective for chicken or bird have been shown to multiply without difficulty on or in the embryo. Since attempts to propagate viruses of plant origin on this living animal medium have not yet been reported, it is the purpose of this paper to describe an experiment in which tobacco mosaic virus 1 has been used to inoculate the chorio-allantois.

Leaves from Turkish tobacco plants showing typical tobacco-mosaic symp-

<sup>1</sup> Jones, L. R. and W. W. Gilbert. Lightning injury to herbaceous plants. *Phytopath.* 8: 270-282. 1918.

<sup>2</sup> Walker, J. C. Injury to cabbage by lightning. *Phytopath.* 27: 858-861. 1937.

toms were frozen, passed through a meat grinder, and the juice expressed by wringing the macerated tissue in a piece of cheesecloth. The extracted juice was centrifuged and purified by Stanley's ammonium sulphate-celite method and then passed through a Berkefeld "V" filter. Fresh fertile chicken eggs were incubated for 9 days at 40° C. and prepared for inoculation, using Burnet's<sup>1</sup> modification of Woodruff and Goodpasture's<sup>2</sup> method as recommended by Professor Jacob Traum and Miss Miriam Smith of the Division of Veterinary Science.

Five eggs were inoculated by gently swabbing a sterile cotton pellet soaked with the purified virus suspension, over the chorio-allantois. Five other eggs were inoculated in a similar way, but by first touching the wet pellet to a small quantity of carborundum powder before inoculating the membrane. By using the carborundum powder it was hoped that some of the virus would be introduced directly into the cells of the membrane through the abraded surface. After incubating the inoculated eggs for 3 days at 37° C., the exposed portion of chorio-allantoic membrane was removed from the living embryos. A small piece of it about 0.3 cm. square from each egg was used to inoculate a succeeding set of incubated eggs. The remaining piece of each membrane was ground in a porcelain mortar and 2 cc. of distilled water were added to make sufficient volume to inoculate 5 half leaves of *Nicotiana glutinosa*. The other half of each leaf was inoculated with the filtered purified virus. Four successive subplants were made on incubated eggs. In no case were local lesions formed on leaf halves inoculated with the egg membrane material, while opposite halves inoculated with filtered purified virus showed an average of 250 spots per half leaf on *N. glutinosa*.

Under the conditions of these experiments, not only did tobacco-mosaic virus fail to multiply in the chorio-allantoic membrane but active virus could not be detected by the local-lesion method in the membrane on which the original virus inoculation had been made.—WILLIAM N. TAKAHASHI, Division of Plant Pathology, University of California, Berkeley, California.

*Carborundum for Plant-virus Inoculations.*—Following the development of the carborundum method,<sup>1, 2</sup> numerous inquiries relating to the grade and quality of powdered carborundum have been received. It has seemed desirable, therefore, to present more complete information regarding the carborundum that has given satisfactory results during the past 5 years.

Powdered carborundum, 600 mesh, is obtainable in any quantity from Braun-Knecht-Heimann Company, San Francisco, California. When ordering, it is advisable to refer to their stock number 38713. Carborundum in-

<sup>1</sup> Burnet, F. M. A virus disease of the canary of the fowl-pox group. *Jour. Path.* 37: 107-122. 1933.

<sup>2</sup> Woodruff, Alice Miles and Ernest W. Goodpasture. The susceptibility of the chorio-allantoic membrane of chick embryo to infection with the fowl-pox virus. *Amer. Jour. Path.* 7: 209-222. 1931.

<sup>1</sup> Rawlins, T. E. and C. M. Tompkins. The use of carborundum as an abrasive in plant-virus inoculations. (Abstract) *Phytopath.* 24: 1147. 1934.

<sup>2</sup> Rawlins, T. E. and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant-virus inoculations. *Phytopath.* 26: 578-587. 1936.

variably contains moisture upon arrival from the distributor; in this condition the powder is dark gray, and the particles adhere to each other in small to large aggregates. In this condition it cannot be used to advantage in a salt shaker. It is suggested, therefore, that, when received, the lid of the container be removed and the carborundum placed in a dry heat sterilizer at 80° to 90° C. for several hours, or until thoroughly dry. Drying restores the powdery condition and the light gray color of this particular grade of carborundum. If stored in tight-stoppered receptacles, no further drying is necessary.—T. E. RAWLINS and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

### BOOK REVIEW

COUCH, J. N. *The Genus Septobasidium*. 473 pages, including 114 plates, 60 text figs. The University of North Carolina Press (Chapel Hill). 1938.

The pathologist encountering this thorough and significant monograph probably will begin with the Pathological Considerations and Control Methods of Chapter 2. Here his interest will be aroused at once by the statement that all species of *Septobasidium* cause damage to the trees on which they grow, the damage, with the extent of growth, varying from considerable to slight, numerous small trees of *Fraxinus* and *Nyssa*, for example, being killed outright by *Septobasidium pseudopedicellatum* and *S. curtisii*, while large trees, heavily infested, are very unhealthy with many dead limbs. Continuing, he will note with interest that the commonest type of injury involves chiefly cracking of the bark, in some cases with girdling of the branches or smaller trunks, and secondarily the formation of witches brooms, such injury occurring not only in the United States, particularly in the South, on such trees as ash, maple, holly, magnolia, pear, apple, etc., but in the American and Oriental tropics on citrus, acacia, tea and others. Reading farther he will note with regret the failure of spraying as a control measure and with approval the success of the direct application of kerosene emulsion paste and of pruning infected branches during the dormant season. Then, as the pathologist reaches the end of the chapter, he will find, to his disappointment, that in Dr. Couch's opinion there is no immediate cause for alarm over *Septobasidium*, for although widespread and although abundant in certain localities, the trees to which it is injurious are not important timber, while on cultivated fruit and nut trees it is serious and abundant only when favored by neglect and poor conditions, not in well kept orchards. Nor will the reader be comforted by the author's final admonition that the *Septobasidium*-scale insect combination must be regarded as distinctly harmful.

At this point it is possible that the young pathologist, conditioned by his previous training to believe that information of value to him must follow the stereotyped pathological pattern with which he is familiar, and inured to a complacent mental myopia, may be inclined to read no farther leaving the remainder of this significant study to the impractical mycologist or the omnivorous entomologist.

Let me assure the young pathologist that in the remaining 476½ pages of this unusually able work he will find that *Septobasidium* offers much of interest and value, even though it may not occasion any remunerative fellowships or become the objective of well financed state or federal campaigns of eradication. The older pathologist does not need this assurance for he has long since realized that basically underlying the practical aspects of plant pathology are such fundamental problems as parasitism and interaction, problems of biological significance in themselves and often of practical value in their bearing on control.

Of especial interest is the well worked out study of the intricate Fungus Insect Relationship in Chapter 1. The scale insects provide food and a means of distribution for the fungus, while the *Septobasidium* furnishes protection and a home for the insects. Of this two-membered consocium it is the insects that damage the underlying tree by their sucurial tubes, which pierce through the bark to the cambium, the fungus contributing indirectly by fostering the insects, but doing little damage directly since in only a few species may the hyphae penetrate slightly into the living tissue of the bark or leaf. The relation involves an unusual nicety of balance, the insect colony, through the negligible sacrifice of the relatively few individuals parasitized by the fungus gaining as a whole an effective protection and a specialized housing, which fosters the production of offspring and facilitates their escape to accomplish the dispersal of the fungus by carrying its spores. The association is thus one of mutual benefit, a symbiosis in the

strict sense of the word, and the biological situation involved, an intersection of mycology, entomology and plant pathology, leads into such fascinating paths of speculation as the evolutionary origin of such a specialized association so obviously of long standing. Furthermore, this combination of insect and fungus results in a new type of organism differing from either participating entity by itself and hence leads into the realm of emergent evolution in the borderland of philosophical biology remote from most of us but explored to the enrichment of biologic thought by such intrepid souls as Wheeler.

The associative complex here involves a large number of scale insects, the 54 species identified from United States material and the 45 known from other sources, belonging chiefly to the genus *Aspidiotus*, some to *Chionaspis* and *Chrysomphalus*, with a few scattered through several other genera, probably representing only a part of the actual representation. The fungous participant, *Septobasidium*, comprises around 170 species of which about half have been established by Couch in his thorough twelve years' study of living and herbarium material in this country, Europe, and the West Indies.

Especially interesting and valuable is the discussion of the geographic distribution of *Septobasidium* so far as it is known, listing the number of species collected from various countries and interpreting both the general distribution and some of the more notable special cases on the basis of such chief means of dissemination as the spread of infected young scale insects, whether through their own crawling or through being carried by birds, by other insects or by the wind, and the spread of established *Septobasidium*-insect communities through transplantation or long distance transport of the host trees. Of interest also is the detailed analysis of the distribution of *Septobasidium* in the United States and Canada with its effective tabulations of distribution by States according to the number of collections not only for the several states but also for the various host trees and its analyses of the special points of interest involved.

Especially thorough and significant also is the detailed account of the structure, development, and reproduction of the fungus with a careful comparative study of the specialized features of the traps, tunnels, houses, and breeding chambers fostering the insects and of the haustoria parasitizing them.

With this comprehensive study as a foundation, the structural features of taxonomic importance are carefully evaluated serving as a sound basis for the 230 pages of the taxonomic section with its revised and extended generic description, its effective key to the species, its detailed specific descriptions and its supplementary notes on species incompletely known or justifiably excluded.

The relation of *Septobasidium* to other members of the lower Basidiomycetes with transversely septate basidia and especially to the rusts is carefully considered and the genus established as an order, Septobasidiales, of the Hetero-basidiomycetes, coordinate with the Auriculariales, Uredinales and Ustilaginales. Questions of cytology and hybridization are covered briefly but adequately, while a comprehensive reference list of 120 titles, thoroughly covering the literature and a serviceable and extensive index complete the text.

The excellent and numerous illustrations fittingly complement the thorough and comprehensive text. Assembled in the 48 half-tone plates are numerous photographs showing the characteristic habit and distinctive gross structural features of all the more important species, while the zinc cuts, wash drawings, and photographs of the frontispiece, the 60 text figures and the first 66 plates illustrate with admirable effectiveness the structure of the participating insects themselves, their development in relation to the host trees and to the traps, breeding chambers and other specialized structures of the fungus, and the context, hymenium, basidia, spores, and other distinctive morphological details of the several species of *Septobasidium*.

The book is a monograph worthy of a place beside that of Thaxter, an inspiration to the mycologist and plant pathologist, a significant contribution to biology as a whole.

\* \* \* \* \*

Since this review was written, Dr. Couch's work has been awarded the Walker Prize of the long established Boston Society of Natural History, a signal honor, as this prize is given in recognition of the most outstanding contribution, whether a single piece of work or a concerted program, in the field of Natural History during the preceding five years.—WILLIAM H. WESTON, Biological Laboratories, Harvard University, Cambridge, Mass.



# THREE SPECIES OF PYTHIUM ASSOCIATED WITH ROOT ROTS

CHARLES DRECHSLER

(Accepted for publication October 7, 1939)

In a paper (9) published nearly 10 years ago I presented as new 15 species of *Pythium* that had been isolated from decaying parts of various phanerogamic host plants originating from different localities in eastern and southern regions of the United States. Aside from some introductory comments, mainly of a comparative nature, the descriptions then accorded to the new forms were limited to diagnostic statements not accompanied either by needful explanatory remarks or by figures illustrative of details and peculiarities difficult to set forth adequately in words. It is hoped that as far as 6 of the species are concerned, these deficiencies of treatment have in a measure been remedied in supplementary accounts (12, 13) that have recently appeared in this journal. Similar supplementary consideration is herein devoted to 3 additional species, *P. dissotocum*, *P. periilum*, and *P. paroecandrum*, all of which I described from pure cultures isolated through procedure elsewhere (7, p. 310-312) recorded, from softened or discolored tissues of roots affected by decay. To facilitate comparison, the accompanying figures were prepared for reproduction at magnifications uniform with those in similar illustrations of the congeneric forms dealt with earlier. As the zoosporangia of *P. paroecandrum* are of the conveniently compact subspherical type and appear unaccompanied by significant differentiation of supporting hyphae, they are shown, like some of the less rangy homologous structures (12, p. 399, fig. 3, I, J, K) of *P. acanthicum* Drechsl., at the same magnification (*i.e.*,  $\times 1000$ ) employed in illustrations of sexual apparatus, rather than at the lower magnification (*i.e.*,  $\times 500$ ) resorted to in figures of the more extensive filamentous or lobulate sporangial units of numerous related species.

## PYTHIUM DISSOTOCUM

The diagnosis of *Pythium dissotocum* Drechsl. was based primarily on a culture submitted to me in a varied assortment of fungus cultures isolated by R. D. Rands from diseased roots of sugar cane, *Saccharum officinarum* L., collected near Thibodoux, Louisiana, in April, 1927; some utilization, however, being made also of observations on 5 other cultures in the same assortment that were easily recognized as conspecific from a thoroughgoing similarity of macroscopic appearance, and from a close parallelism in mycelial habit, zoosporangial development, and arrangement of sexual apparatus revealed by each of them under the microscope. On diseased sugar-cane roots the fungus would seem to occur with moderate frequency. Under the binomial *P. dissotocum*, Rands and Dopp (19) make mention of 57 cultures that were isolated by them from such roots and subjected to growth measurements and to tests for pathogenicity. They further cite *P. dissoto-*

*cum* among the 3 species that, apart from *P. arrhenomanes* Drechsl., were most frequently obtained by them from rotted sugar-cane roots in 1930. Inoculation experiments of these authors reveal the fungus as only weakly parasitic when environmental conditions are in ordinary degree favorable for the host plant; severe root rot with appreciable reduction in plant weight ensuing, however, under the predisposing influence of a soil toxin, salicylic aldehyde, in dilute concentration.

The fungus is known to occur also on phanerogamic plants other than sugar cane. It was found in several sets of cultures isolated from softened roots of canning peas, *Pisum sativum* L., collected in the course of a survey on which a report (5) was rendered in 1925. The sets of cultures in question were derived from collections made respectively near Easton, Maryland, May 15, 1924; near Centerville, Md., May 16, 1924; near Bridgeville, Delaware, May 27, 1924; near Cedarville, New Jersey, May 29, 1924; and near Westminster, Md., June 11, 1924. *Pythium dissotocum* was likewise represented in 2 cultures isolated by F. R. Jones from pea roots collected in the course of another disease survey (16). One of these cultures was derived from material collected near Templeton, Wisconsin, July 4, 1924; the other from material collected near Gillett, Wis., July 17, 1924. The fungus was obtained later from discolored rootlets of *Pilea pumila* (L.) Gray collected near Cabin John, Md., Oct. 20, 1926. Four cultures isolated from discolored rootlets of the sugar beet, *Beta vulgaris* L., gathered near Saginaw, Michigan, late in June, 1927, have been readily identified as belonging to the species; and a similar determination was made of 3 cultures derived from discolored roots of spinach, *Spinacea oleracea* L., collected near Norfolk, Virginia, late in November, 1932.

In pure culture on a transparent gel substratum not excessively rich in nutrients, such as is available more especially in maize-meal agar, *Pythium dissotocum* grows appreciably more slowly than the very familiar congeneric forms causing damping-off in seed-beds. Aerial mycelium is usually altogether absent on this medium, though sometimes developing in meager quantity on substrata that contain food substances in higher concentrations. As was stated in the diagnosis, the submerged mycelium is somewhat lustrous, presenting an appearance, therefore, in a sense median between that of the diffuse intramatrical mycelium of *P. ultimum* Trow on the one hand, and that of the very lustrous mycelium of *P. complens* Fischer on the other. Commensurate with its moderate luster, the thallus of *P. dissotocum*, while consisting more largely than that of *P. ultimum* of rather straightforward axial hyphae arranged nearly parallel with one another, is composed of such hyphae in smaller measure than is the thallus of *P. complens* or of the similarly very lustrous *P. vexans* de Bary (= *P. complectens* Braun). The curious regional variegation with respect to density of hyphal development that becomes apparent to the naked eye in a cumulous effect is not usually evident in cultures of the fungus under consideration. Appressoria of modest

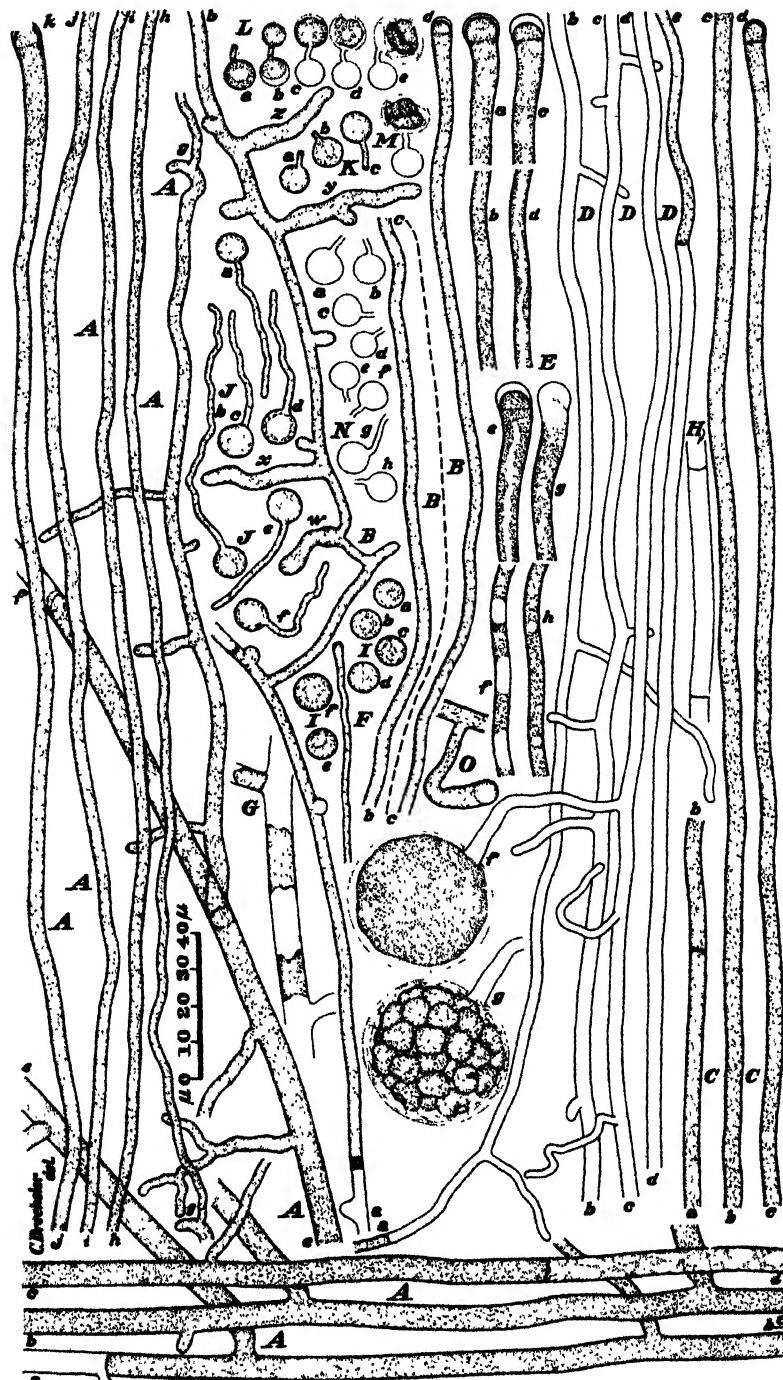


FIG. 1. Asexual reproductive apparatus of *Pythium dissotocum* Drechsl. drawn with aid of a camera lucida;  $\times 500$ . From lack of space A is shown in sections connecting at points b, c, e, g, h, i, j; B similarly connecting at points b, e; C connecting at points b, c; and D connecting at points b, c, d.

dimensions (Fig. 1, O) are formed in some numbers where hyphae come in contact with the surface of the culture dish or of other hard objects.

Production of zoospores by *Pythium dissotocum* ensues with much regularity when vigorous mycelium of the fungus is bathed in water. Extraordinarily prolific development of swimming spores may be induced conveniently by cutting sizeable slabs well permeated with young mycelium from a vigorously growing Lima-bean agar plate culture, then transferring the excised slabs to an empty sterile Petri dish, and irrigating them by careful addition of well-aerated sterile water until the upper surface of the substratum is barely flooded. Often, on proper manipulation, virtually the entire mycelium becomes converted into sporangial units. In many instances the individual unit is composed of a longish portion of a wide axial filament together not only with contiguous portions of a few main branches but also with perhaps more numerous narrower lateral ramifications, one of which may become prolonged into a rangy evacuation tube more than 1 mm. in length (Fig. 1, A, a-k). Such a large extensive sporangium naturally gives rise to a correspondingly large vesicle wherein from 100 to 125 zoospores may be fashioned. A sporangial unit of more moderate volume consists often of an intercalary portion of filament, 1 to 2 mm. long and 3 to 4  $\mu$  wide, together with a half-dozen subsidiary ultimate branches, and yields between 50 and 75 zoospores (Fig. 1, D, a-e, f, g). Frequently an unbranched terminal portion of filament, from 0.5 mm. to 1 mm. in length, becomes delimited by a basal septum, and, after forming a broad tip of dehiscence (Fig. 1, C, a-d), functions as a sporangium. A small sporangium may be provided with an evacuation tube measuring as little as 1.5  $\mu$  in width below an expanded tip only 2.5  $\mu$  wide (Fig. 1, F).

Under conditions very favorable for zoospore production, conversion of a portion of vegetative mycelium into an asexual reproductive unit is in most instances not preceded or accompanied by development of any specially differentiated elements, apart, of course, from the expanded cap of dehiscence. In less numerous instances (Fig. 1, B, a-d), however, such conversion entails production of several swollen lateral branches (Fig. 1, B, w-z) noticeably wider than the filament bearing them, though frequently not exceeding in width the undifferentiated main axial hyphae of the fungus. The swollen branches attain more conspicuous development ordinarily after an expanse of mycelium has largely exhausted itself in zoospore production and has possibly been affected besides by some accumulation of staling products, as well as by incipient bacterial contamination (Fig. 2, A). Though usually affording only a rather meager display, the daetyloid branches appear truly homologous with the distended digitations, lobulations, and moriform aggregations familiar in certain congeneric forms like *Pythium arrhenomanes*, *P. complens* and *P. periplocum* Drechs.

The zoospores of *Pythium dissotocum*, after swimming about very actively for a variable period, come to rest and round up, thereby forming spherical cysts slightly smaller than the cysts of *P. debaryanum* Hesse and of most

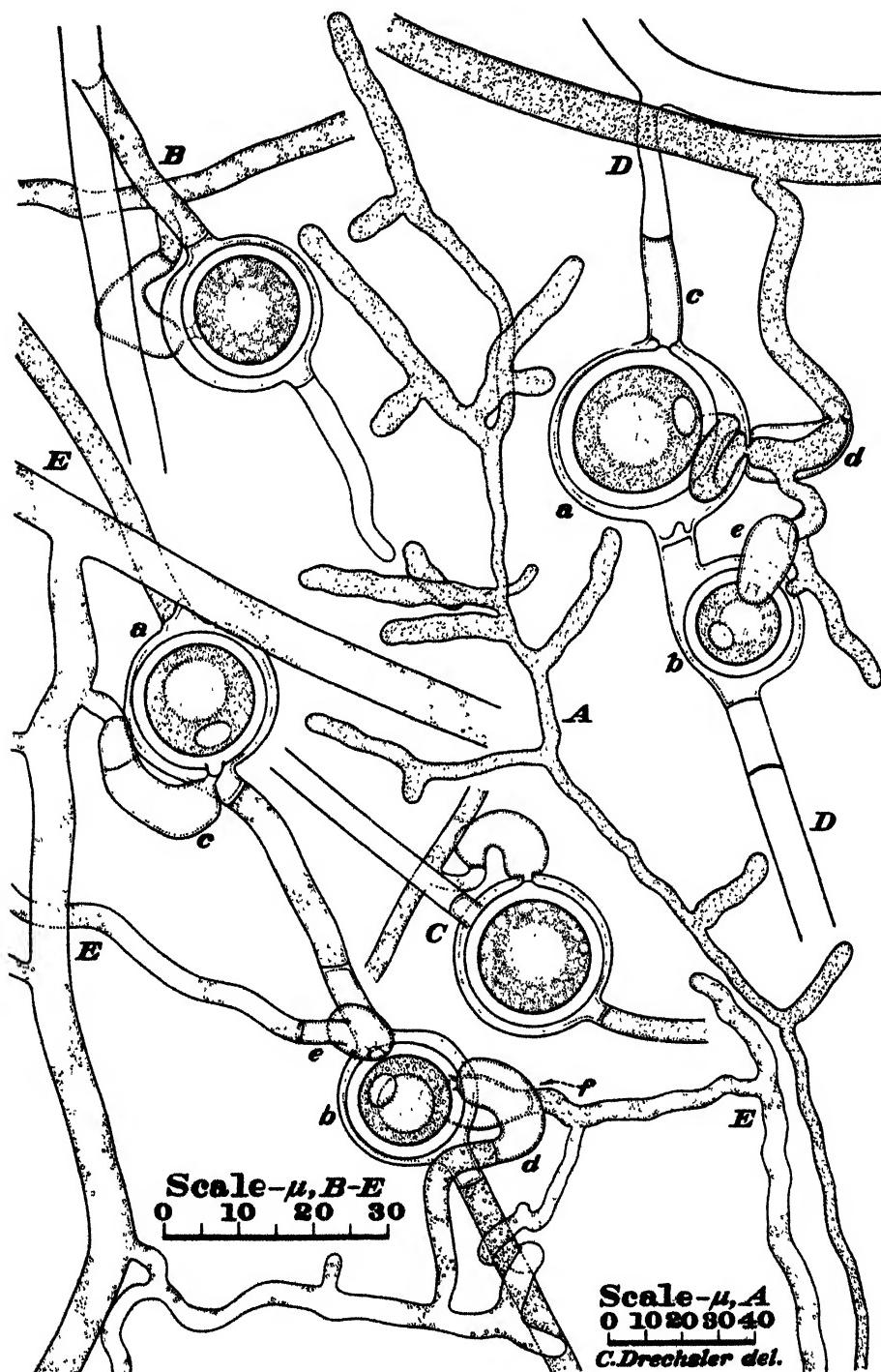


FIG. 2. *Pythium dissotocum* drawn with the aid of a camera lucida. A. Portion of irrigated mycelium, showing zoosporangial differentiation in relatively luxuriant development of inflated lateral branches;  $\times 500$ . B-E. Units of sexual apparatus;  $\times 1000$ .

Scale -  $\mu$ , A  
0 10 20 30 40  
C. Drechsler del.

other allied species familiar to plant pathologists. Often the spherical bodies then germinate in a commonplace manner by the production usually of a single delicate germ hypha (Fig. 1, J, a-f); or, again, they may develop iteratively, each putting forth an evacuation tube (Fig. 1, K, a-c; L, a) and discharging its contents (Fig. 1, L, b) into a small vesicle, there to be fashioned into a new biciliate motile zoospore (Fig. 1, L, c-e; M) of the same type as the one from which it originated. As a result of such iterative development, innumerable empty cyst envelopes with open evacuation tubes of varying lengths (Fig. 1, N, a-h) are often to be observed scattered about everywhere in an irrigated preparation.

The frequency of iterant swarming in zoospores of *Pythium dissotocum*, to which brief allusion was made in an earlier paper (8, p. 569, lines 47 to 50) devoted mainly to similar activity in zoospores of various other pythaceous forms, suggested the specific epithet, a term meaning "twice-born," subsequently applied to the fungus. Additional instances of iterant swarming following repeated emergence have since been supplied by Sparrow (21) in the descriptions of his *P. adhaerens* and his *P. angustatum*. Höhnk (14) noted that the zoospores of the fungus he described as *P. epigynum* underwent a second swarm period when fresh water was added after a first encystment had occurred. This investigator later took occasion to give details concerning particular examples of repetitional development observed by him (15).

Most strains of *Pythium dissotocum* ordinarily show abundant and prompt sexual reproduction when grown on maizemeal agar containing in suspension a moderate quantity of finely ground maizemeal. It is true, sexual reproduction occasionally fails to take place, not only in the more refractory strains but also in strains habitually productive of oospores in immense numbers. As the conditions evoking such apparently capricious behavior have not hitherto been determined, it may only be conjectured that possibly some specific nutrient substance, not always available in sufficient quantity, exerts a governing influence. However, once sexual structures have been formed, they are little given to degeneration on a serious scale.

The subspherical oogonia of the fungus may be terminal (Fig. 3, A; D, a; H) or subterminal (Fig. 2, B; Fig. 3, E) on branches of variable length, though more often they are borne on the main hyphae in intercalary positions, sometimes mesially (Fig. 2, C; D, a; E, a, b; Fig. 3, B; C; D, b; F; J; K, b; L, a) or, again, laterally (Fig. 3, G, a, b; I; K, a; L, b). As the delimiting septa are often placed at appreciable distances from the subspherical contour, cylindrical parts, commonly 2  $\mu$  or 3  $\mu$  long, but sometimes measuring 5  $\mu$  (Fig. 3, J), 6  $\mu$  (Fig. 3, C), 7  $\mu$  (Fig. 3, D, b) or even 11  $\mu$  (Fig. 2, E, b) in length, come to be included in the female organ. Not infrequently, 2 intercalary oogonia are formed adjacent to each other (Fig. 2, D, a, b; Fig. 3, G, a, b; L, a, b).

The male complement of the individual oogonium consists usually of 1 to 3 antheridia, which, for the most part, are of the inflated crook-necked type

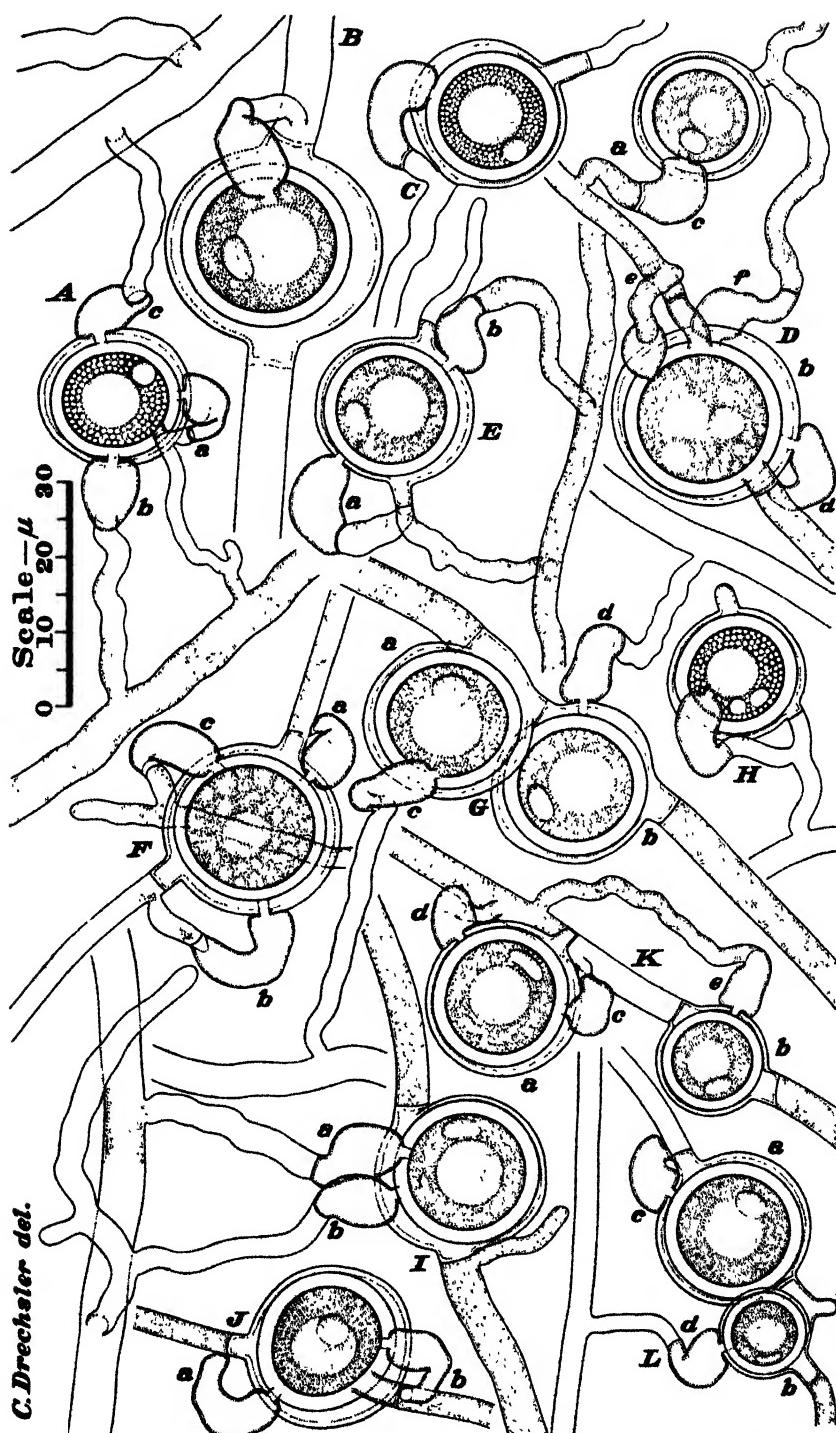


FIG. 3. Sexual apparatus of *Pythium dissotocum* drawn with the aid of a camera lucida to a uniform magnification;  $\times 1000$  throughout.

familiar in many congeneric species. Where plural antheridia are present they appear usually to have arisen without reference to one another, often despite a moderately close mycelial connection between them. As for their other relationships, the antheridia are often borne terminally on branches arising either from neighboring hyphae having no close connection with the oogonial filament (Fig. 2, B; C; D, e; E, e, d, f; Fig. 3, A, c; D, c; F, c; G, c, d; I, a, b; L, d), or from the oogonial filament at variable distances from the oogonium (Fig. 3, A, b; E, b; H; K, c, d, e), or often, again, from the oogonial filament in close proximity to the oogonium (Fig. 3, A, a; B; C; D, e; E, a; F, b). Often, too, an antheridium is borne sessile on the oogonial filament in immediate proximity to the oogonium (Fig. 3, D, d; F, a; J, a, b; L, c). The "hypogynal" type of antheridium, consisting of an outwardly undifferentiated portion of filament adjacent to the oogonium (Fig. 2, D, c) has likewise been observed in cultures of the fungus, though so infrequently that the few instances must be regarded as somewhat exceptional.

Application of the antheridium to the oogonium usually entails some apical flattening, so that contact between the opposed organs is generally wider in this species than in *Pythium perillum*, for example. In units of sexual apparatus with plural antheridia, all ordinarily discharge their contents into the oogonium. As in many other fungi, various irregularities of sexual reproduction occur in *P. dissotocum*, an example being illustrated somewhat incidentally in figure 2, D, where the antheridium "d," which presumably became applied to the oogonium "a" at a relatively late stage, is shown to have resorted to vegetative growth by intruding a hyphal prolongation into the unoccupied portion of the oogonial chamber, and by putting forth laterally a filament that gave rise to a short branch bearing the antheridium "e," which clearly was operative in the fertilization of the oogonium "b."

Following fertilization the oogonium of *Pythium dissotocum* shows a sequence of internal change familiar in most congeneric species. The contracted protoplast surrounds itself with a thick wall. The sizeable lumps of somewhat homogeneous consistency into which the porridge-like granular material has been aggregated, become displaced at the center of the young sexual spore by a homogeneous globule of increasing size (Fig. 2, B; C; Fig. 3, F). The resulting parietal layer diminishes in thickness correspondingly, and often reveals a perceptibly radial orientation as its constituent lumps undergo transformation into smaller granules (Fig. 3, D, a, b; E; G, a, b; J). At early maturity the layer has a densely and rather minutely granular texture, contrasting sharply with the apparently homogeneous structure of the single subspherical or oblate ellipsoidal refringent body (Fig. 2, D, a, b; E, a, b; Fig. 3, B; I; K, a, b; L, a, b). After several weeks of aging it is often found composed of larger subspherical granules measuring about .5  $\mu$  in thickness (Fig. 3, A, C), and the single refringent body may be replaced by 2 similar bodies of slightly reduced dimensions (Fig. 3, H).

In the texture of its parietal layer the oospore at advanced maturity thus comes to present an engaging resemblance to the oospores of various Saprolegniaceae, including the several terrestrial parasitic species known to cause root rot in phanerogamic crop plants. This resemblance would seem sustained in a transitory arrangement of protoplasm observable in the zoosporangium of *Pythium dissotocum* shortly, though not immediately, preceding its evacuation. During the earlier stages in the development of an apex of dehiscence (Fig. 1, E, a), the contents of the hyphae to be included in the new sporangial unit show little alteration from the granular texture usual in vegetative filaments (Fig. 1, E, b). Later, when the hyaline cap has nearly attained its definitive proportions (Fig. 1, E, c), longitudinal vacuoles make their appearance in the hyphae, and often coalesce into extended axial lacunae of irregular outline (Fig. 1, E, d). In some of the narrower filamentous elements, though not usually throughout the sporangium (Fig. 1, E, e), the longitudinally vacuolate condition may for a brief time be supplanted by a transversely vacuolate one (Fig. 1, E, f), so that cylindrical portions of protoplasm alternate with regularly spaced vacuoles in a manner recalling the arrangement of zoospore protoplasts in *Aphanomyces* sporangia previous to their becoming connected by axial strands. To be sure, the transversely vacuolate condition is rather completely obliterated in the sudden reorganization of contents that precedes sporangial discharge by a few seconds. In this reorganization, which is often accompanied by a visible jolt of the hyphae concerned, the protoplasmic contents are withdrawn a short distance from the hyaline cap (Fig. 1, E, g), and revert throughout the reproductive unit to a granular texture relieved only sparingly by a few small, scattered vacuoles (Fig. 1, E, h).

An interrupted disposition of protoplasm, somewhat similar to that associated transitorily with sporangial development, is observable often in aging vegetative filaments (Fig. 1, G) of the fungus. Although aging of mycelium here entails much less abundant deposition of retaining cross-walls than in *Pythium debaryanum*, for example, successive septa may occasionally be found rather closely spaced in the empty hyphae (Fig. 1, H).

With regard to its principal dimensions, *Pythium dissotocum* shows the rather moderate range of variability prevailing in most members of the genus to which it belongs. The data in the diagnosis relevant to oogonial size were derived from 200 measurements of mature oogonia of obviously wholly normal development selected at random in maize-meal-agar cultures showing very copious sexual reproduction with virtually no degeneration. The 200 values for diameter of oogonium, expressed to the nearest micron, showed a distribution as follows: 12  $\mu$ , 1; 14  $\mu$ , 1; 15  $\mu$ , 3; 16  $\mu$ , 1; 17  $\mu$ , 3; 18  $\mu$ , 12; 19  $\mu$ , 34; 20  $\mu$ , 33; 21  $\mu$ , 43; 22  $\mu$ , 36; 23  $\mu$ , 17; 24  $\mu$ , 6; 25  $\mu$ , 7; 29  $\mu$ , 2; 32  $\mu$ , 1. Measurements of the 200 oospores of correct structure contained within the oogonia, gave the following values for diameter, expressed to the nearest micron: 11  $\mu$ , 2; 12  $\mu$ , 1; 13  $\mu$ , 1; 14  $\mu$ , 2; 15  $\mu$ , 8; 16  $\mu$ , 25; 17  $\mu$ , 48; 18  $\mu$ , 45; 19  $\mu$ , 42; 20  $\mu$ , 14; 21  $\mu$ , 6; 22  $\mu$ , 4; 26  $\mu$ , 1; 27  $\mu$ , 1.

## PYTHIUM PERIILUM

The same collection of fungus cultures from sugar-cane roots that supplied the material on which primarily was based the description of *Pythium dissotocum*, included also the single culture from which, after varied treatment and propagation, was drawn the diagnosis of *P. periilum* Drechs. Subsequently, a number of additional cultures, closely similar to the one in question with respect to macroscopic appearance, as well as with respect to morphology of sporangium and sexual apparatus, were committed to me by R. D. Rands and E. Dopp, who had isolated them likewise from affected roots of sugar cane in Louisiana. These investigators have found the fungus only feebly aggressive as a parasite, for under experimental conditions permitting severe damage by *P. arrhenomanes*, it caused only occasional root tips to become flaccid (19). In commenting on *P. periilum*, Stevenson and Rands (22) characterize the species as a weakly parasitic one, infrequently isolated from rotted rootlets of sugar cane.

On maize-meal agar, *Pythium periilum* shows approximately the same rate of hyphal extension as *P. dissotocum*, and produces similarly an intramatrical mycelium with a lustrous radiate appearance expressive of a considerable degree of parallelism in the orientation of the main axial filaments. However, instead of the relatively uniform mycelial distribution usual in cultures of *P. dissotocum*, the vegetative thallus has rather marked local inequalities in the concentration of its hyphae, whereby it offers to the naked eye a patchy effect that from a suggestiveness of banked cumulous clouds was denominated "cumulous" in the diagnosis of the species.<sup>1</sup> Though aerial growth is usually absent in cultures on maize-meal agar, some richer substrata as, for example, Lima-bean agar, sometimes afford meager development of aerial mycelium in a somewhat appressed, compact, felty layer.

On microscopic examination of its vegetative mycelium the fungus is revealed as one of the more delicate members of the genus to which it belongs. Knob-like appressoria of relatively small dimensions (Fig. 4, A, B) are formed in moderate numbers terminally on some of the delicate branches that encounter the bottom of a culture dish, or that otherwise come into contact with a hard object.

Asexual reproduction can be induced conveniently in *Pythium periilum* by excising sizeable slabs from a thinly poured Lima-bean-agar plate culture well permeated with young mycelium, and transferring them to a shallow layer of aerated sterile water in a sterile Petri dish. Some reproductive units are formed by direct conversion of undifferentiated filamentous hyphae, with only a rather meager increment accruing through development of an expanded tip of dehiscence. Sporangia of such meager external modification are, however, less frequent in irrigated material of the present fungus than in irrigated preparations of *P. dissotocum*, owing to a more abundant production of swollen digitate elements, here singly, there in some-

<sup>1</sup> Sideris (20) has since made reference to the same macroscopic effect by the descriptive term "rosette."

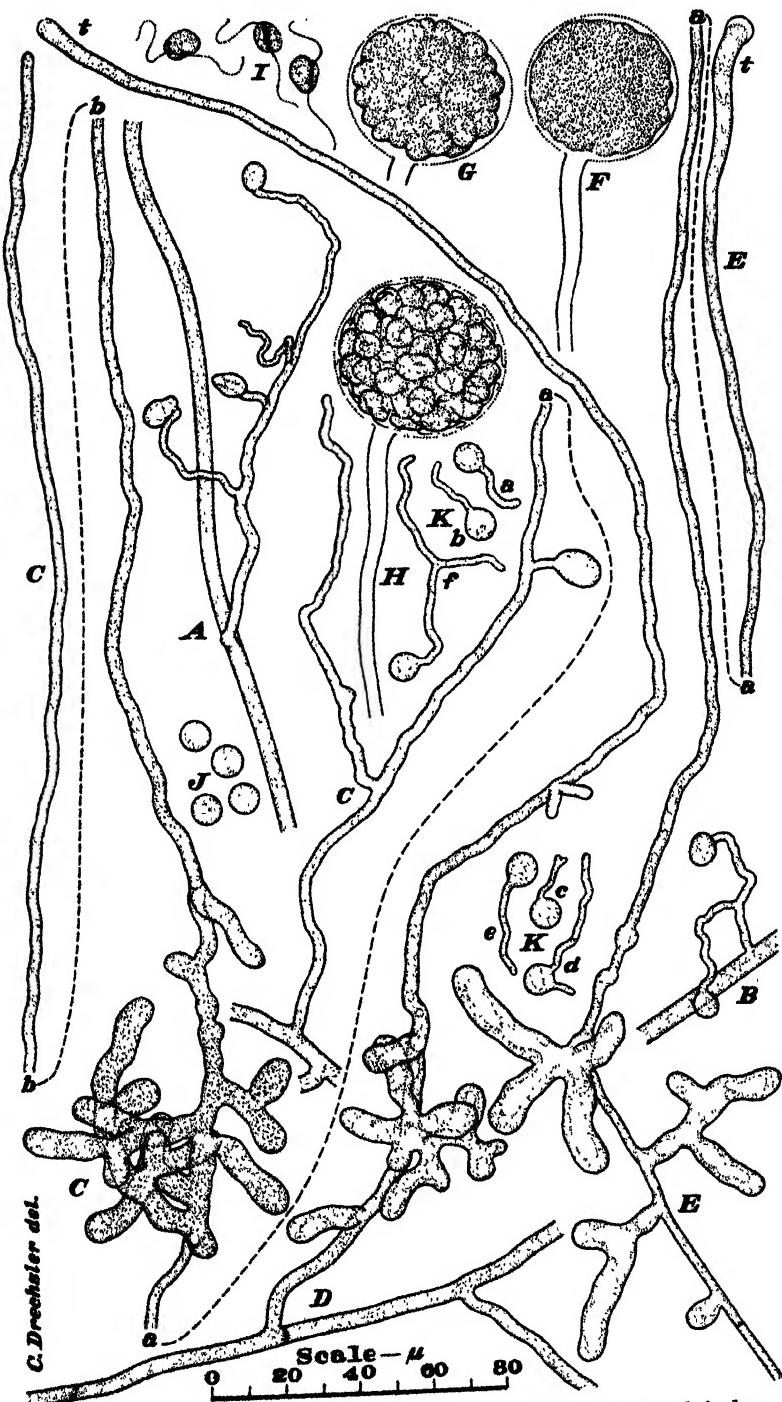


FIG. 4. Asexual reproductive apparatus of *Pythium perulatum* Drechsl. drawn with aid of a camera lucida;  $\times 500$ . From lack of space C is shown in sections connecting at the points a, b; E similarly connecting at the point a.

what intricately branching systems (Fig. 4, C) comparable, more especially perhaps, with the homologous sporangial complexes of *P. myriotylum* DrechsL. Collectively, the swollen parts included in a sporangium, when it comes to be delimited by deposition of a septum (Fig. 4, E) or of plural septa (Fig. 4, D), often are of a volume equal to (Fig. 4, D) or exceeding that of the unmodified filamentous parts. As in other species vacuolization of the associated filamentous and swollen elements proceeds simultaneously with the development of a widened refringent apex of dehiscence on an evacuation tube frequently of considerable length (Fig. 4, D, t; E, t). Discharge of the sporangial contents into a vesicle resulting from inflation of the refringent cap (Fig. 4, F), transformation of the discharged mass into biciliate zoospores (Fig. 4, G, H), and liberation of the motile bodies (Fig. 4, I) by disintegration of the vesicular membrane, follow in familiar sequence.

With appropriate irrigation *Pythium periilum* produces swarm spores in about the same moderate measure of abundance as *P. myriotylum*; the fungus being in general more prolific asexually than *P. debaryanum*, *P. irregularare* Buism., and *P. mammillatum* Meurs, but appreciably less prolific than *P. butleri* Subr., and decidedly less prolific than *P. dissotocum*. After swimming about for some time the zoospores come to rest and round up into cysts (Fig. 4, J) that like the cysts of *P. dissotocum* would seem to be somewhat smaller than the homologous bodies of most of the congeneric parasites causing damping-off. Germination of the globose structures takes place mostly by production of a delicate germ tube (Fig. 4, K, a-c, e, f) or of 2 such tubes (Fig. 4, K, d).

In maize-meal agar cultures, containing some finely divided maize-meal, *Pythium periilum* gives rise promptly to sexual apparatus that develops usually with little evidence of degeneration. The subspherical oogonia appear very often in intercalary positions (Fig. 5, A, D-L), less frequently in subterminal (Fig. 5, B) or terminal positions (Fig. 5, C). Generally, while the individual female organ is still continuous with its supporting hypha, it becomes inwrapped rather intimately and extensively by a branching filament (Fig. 5, A). Usually this filament arises from a hypha having no close mycelial connection with the one bearing the oogonium (Fig. 5, A-G, I-K), but occasionally it originates as a branch given off by the oogonial hypha at a distance perhaps not exceeding 50  $\mu$  from the female organ (Fig. 5, H). On the ramifications of this filament are soon borne, mostly terminally, but in some cases approximately laterally, from 2 to 5 antheridia, which become delimited by basal septa at about the same time the oogonium also is demarcated by deposition of one or, more often, 2 cross-walls, now flush with the spherical contour, now at a distance of 1 to 4  $\mu$  from it (Fig. 5, B-L). As a rule all of the antheridia discharge their contents into the oogonium, whereupon, if degeneration does not intervene, an oospore is formed that at early maturity shows the unitary internal organization evident in ripe oospores of most species of *Pythium*,—its single

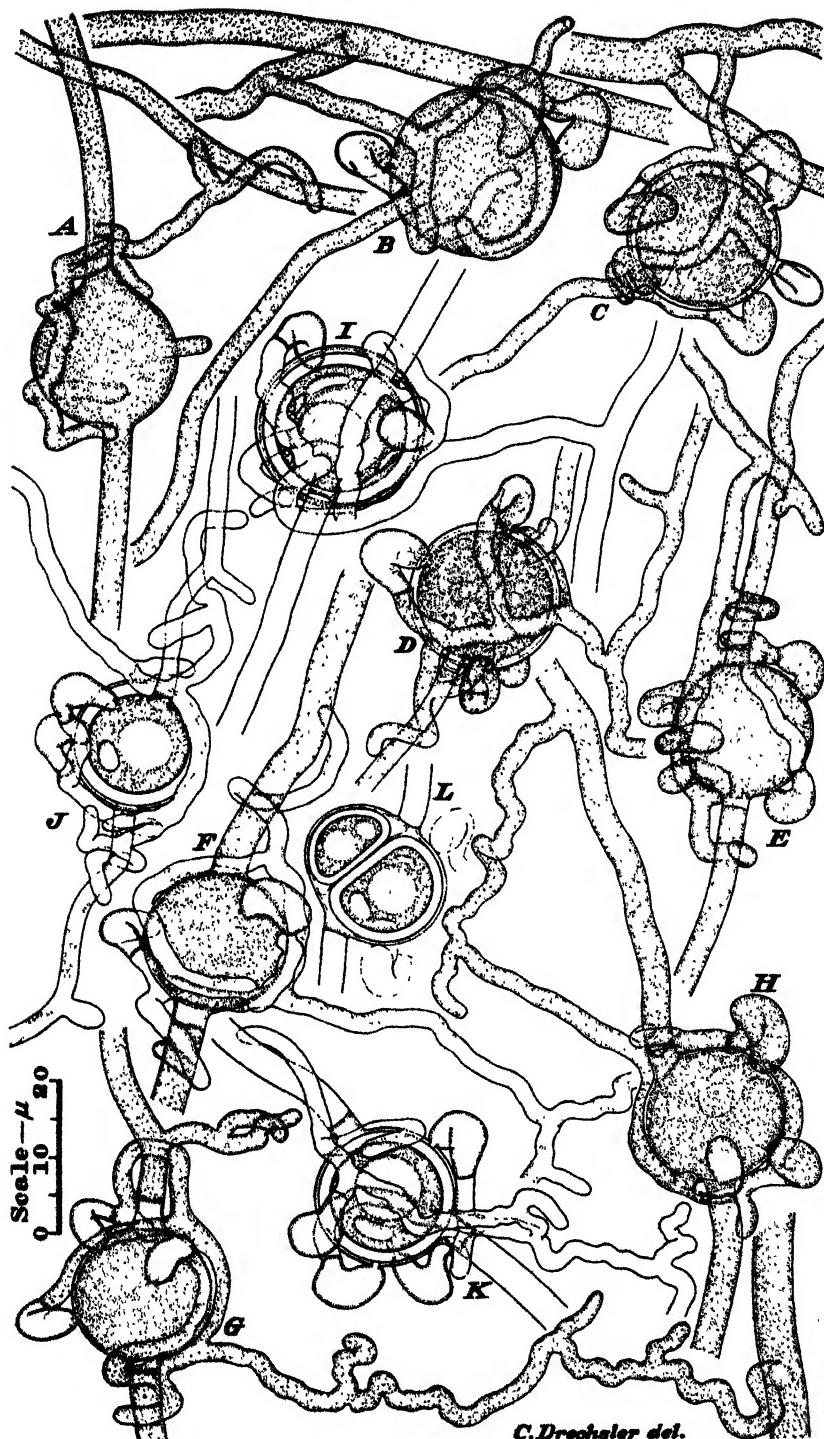


FIG. 5. Sexual reproductive apparatus of *Pythium perulum* drawn with the aid of camera lucida to a uniform magnification;  $\times 1000$  throughout.

central reserve globule being surrounded by a densely granular parietal layer in which is imbedded a single refringent body, subspherical or oblate-ellipsoidal in shape (Fig. 5, I-K). Developmental irregularity sometimes becomes manifest in the production of 2 oospores within an oogonium (Fig. 5, L). For better comparison with other species, all inordinately fecund units of sexual apparatus were excluded from consideration in the parts of the diagnosis relevant to the main dimensions of oogonium and oospore; the data there given having been derived from measurements of 100 monosporous sexual units selected at random in a maizemeal-agar culture that had produced oospores very abundantly with little evidence of degeneration. The 100 oogonia gave values for diameter, expressed to the nearest micron, with a distribution as follows: 16  $\mu$ , 1; 17  $\mu$ , 13; 18  $\mu$ , 23; 19  $\mu$ , 31; 20  $\mu$ , 22; 21  $\mu$ , 7; 22  $\mu$ , 3; and the oospores of correct unitary internal structure contained within them gave values for diameter, likewise expressed to the nearest micron, with the following distribution: 14  $\mu$ , 1; 15  $\mu$ , 2; 16  $\mu$ , 21; 17  $\mu$ , 32; 18  $\mu$ , 26; 19  $\mu$ , 14; 20  $\mu$ , 4.

Rather little diagnostic value attaches to the sizes of oogonium and oospore in *Pythium periilum*, as the homologous bodies of many congeneric forms, including the several species most commonly implicated in damping-off, reveal approximately similar dimensions. Certainly, much greater distinctiveness is represented in the extensive and close inwrapment of the female organ by the branching antheridial filament,—this feature, indeed, having suggested the epithet applied to the fungus, a term derived from a word meaning "to wrap round." Inwrapment of equal extent and intimacy, though frequent among terricolous species of *Aphanomyces*, has been encountered elsewhere in the genus *Pythium*, as far as I am aware, only in *P. scleroteichum* Drechsl. (10), the parasite that, with *P. ultimum*, is responsible for mottle necrosis, a curiously labyrinthine decay of the edible roots of the sweet potato, *Ipomoea batatas* (L.) Lam. Similarity to *P. scleroteichum* is recognizable, besides, in a characteristic frail appearance of the rather small, thin-walled antheridia, which, like the thin-walled branches supporting them, become almost indiscernible after being evacuated of contents. However, the transverse dorsal furrowing, often to be seen in the antheridial branches of *P. scleroteichum*, has never been observed in *P. periilum*. Further, in *P. periilum* the oospore so nearly completely fills the oogonial chamber that, often in large part, its relatively thick wall appears more or less closely adnate to the much thinner, somewhat evanescent oogonial membrane; whereas, in *P. scleroteichum*, the oospore is always very loosely contained within a considerably larger oogonium, and its wall only slightly exceeds in thickness the conspicuous and extraordinarily enduring oogonial envelope.

#### PYTHIUM PAROECANDRUM

The diagnosis of *Pythium paroecandrum* Drechsl. was based primarily on a culture isolated from the somewhat blackened tip of a rootlet that alone

seemed to harbor an infection among hundreds of wholly unblemished rootlets on a flourishing clump of field garlic, *Allium vineale* L., originating from near McLean, Virginia, early in May, 1925. The culture in question had been the first one referable to the species to come into my hands. Previous to its description the fungus had been isolated also from several discolored rootlets of the pale touch-me-not, *Impatiens pallida* Nutt., taken from specimens of that plant collected in the District of Columbia early in September, 1926. A few additional conspecific cultures have since been obtained from separate discolored rootlets of the bloodroot, *Sanguinaria canadensis* L., collected in Arlington, Va., in April, 1931.

When planted on maizemeal agar, *Pythium paroecandrum* gives rise to a slightly lustrous intramatrical mycelium of rather pronounced radiate appearance that extends itself about half as rapidly as mycelium of *P. ultimum*, *P. debaryanum* or *P. irregularare*. On this medium the fungus, unlike the 3 congeneric forms mentioned, produces usually no aerial hyphae, although on various richer substrata, such as Lima-bean agar, some meager aerial development may take place. When portions of a vigorously growing culture are removed to a shallow layer of water devoid of nutrients, hyphae are put forth into the surrounding liquid only a short distance and in relatively small quantity. In its feeble extramatrical development the fungus differs markedly from the species habitually associated with damping-off,—the difference connoting undoubtedly an incapacity on the part of *P. paroecandrum* to operate destructively as a seed-bed parasite, inasmuch as strong extramatrical development evidently constitutes not an incidental but an essential and necessary attribute of damping-off pathogens, enabling them to span readily the tracts of unnutritious soil separating individual seedlings from one another.

On appropriate irrigation fresh growth of *Pythium paroecandrum* rather promptly gives rise to subspherical zoosporangia. In dimensions and general conformation these bodies resemble approximately the zoosporangia of *P. debaryanum*, *P. irregularare* and *P. mammillatum*, though perhaps they more frequently include at one (Fig. 6, A, a) or both ends (Fig. 6, B) an outwardly unmodified hyphal portion between 5  $\mu$  and 50  $\mu$  in length. A sporangium with hyphal prolongations here, like the similarly composite asexual reproductive structures frequent in *P. acanthicum*, very often puts forth the evacuation tube from the cylindrical component (Fig. 6, A, t; B, t), rather than from the subspherical part. Indeed, even in instances where a hyphal extension is relatively short and of small volume, it yet serves frequently as origin of the evacuation tube (Fig. 6, C, t; E, t; F, t). The more purely subspherical sporangia usual in the species show little preference for any particular positional relationship of the evacuation tube (Fig. 6, G, t-O, t). With regard to vacuolization of the protoplasm within a sporangium, formation of a somewhat expanded hyaline cap at the tip of the evacuation tube (Fig. 6, B, t), discharge of the granular contents into a vesicle resulting from inflation of the cap, cleavage of the discharged

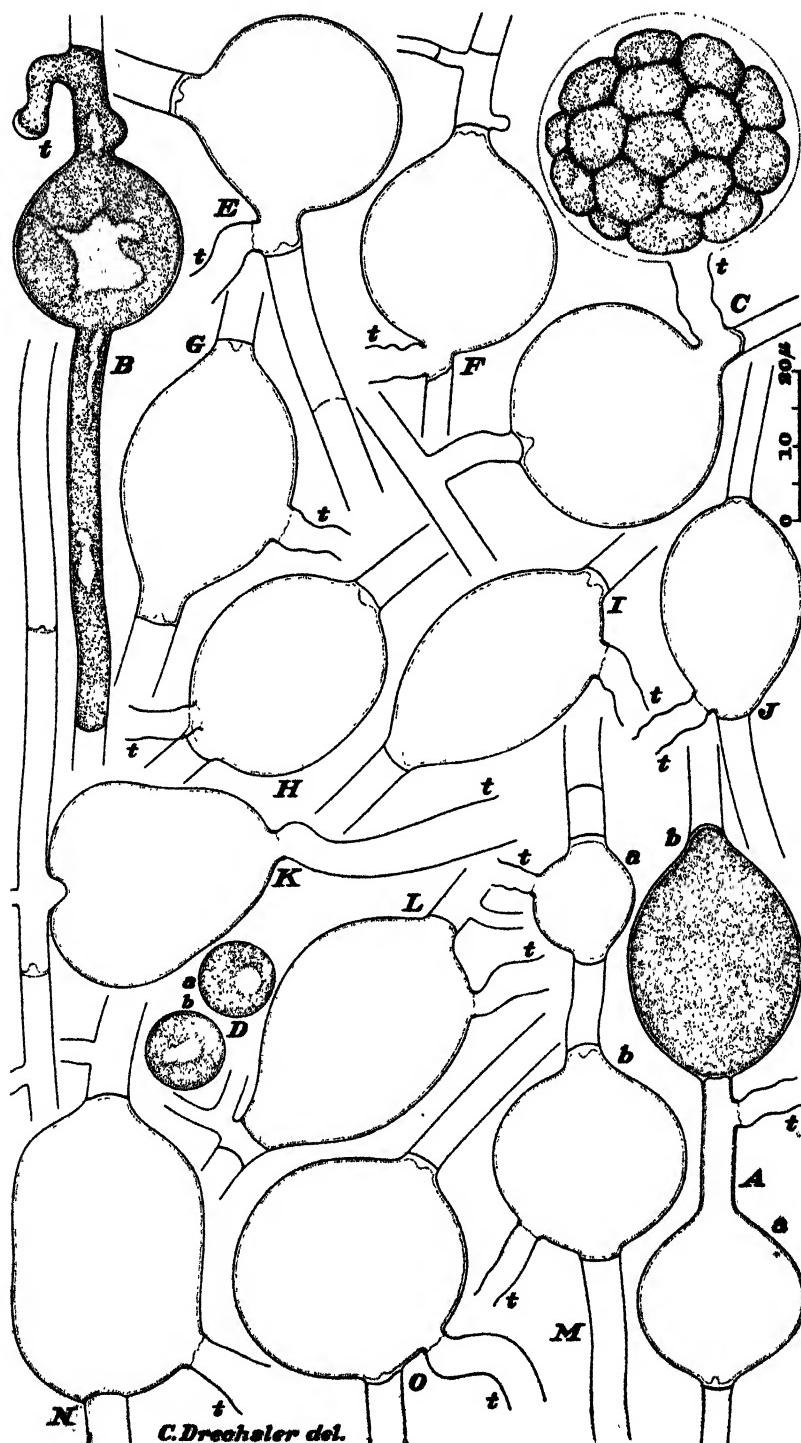


FIG. 6. Asexual reproductive apparatus of *Pythium paroecandrum* Drechsler, drawn to a uniform magnification with the aid of a camera lucida;  $\times 1000$  throughout.

protoplasmic mass (Fig. 6, B), and its transformation into motile biciliate zoospores, the fungus shows general parallelism with *P. debaryanum*, *P. irregulare* and *P. mammillatum*. Its zoospores, like those of the 3 congeneric forms mentioned, round up into globose cysts (Fig. 6, D, a, b) slightly larger than the homologous bodies of *P. dissotocum* and *P. perium*.

In maize-meal agar cultures *Pythium paroecandrum* forms sexual apparatus promptly and abundantly. The oogonia appear comparable to the asexual zoosporangia in their generally subspherical shape and predominantly intercalary position. They are supplied individually with 1 (Fig. 7, J; Fig. 8, A, f, g; B, d; D; E; F; G; I; O, e) to 5 (Fig. 7, C, c-g) antheridia. An antheridium often arises from a hypha having no close mycelial connection with the oogonial filament; but more frequently it originates from the oogonial hypha in close proximity to the oogonium. When of remote origin the male organ may consist of a saccate cell, borne laterally on an axial hypha (Fig. 8, D, I), or of a crook-necked inflated cell, borne terminally on a branch of varying length (Fig. 7, C, e, f, g); in neither case, however, revealing such variety and distinctiveness as when it arises in proximate relationship to the oogonium. The simplest type of antheridium contributed by the oogonial filament consists merely of an outwardly unmodified portion of the filament adjacent to the female organ (Fig. 7, F, a; Fig. 8, G; J, a; K, a). Such an antheridium, sometimes only 10  $\mu$  (Fig. 7, F, a), at other times over 25  $\mu$  (Fig. 8, J, a) long, of necessity thrusts its fertilization tube directly through the septum delimiting the oogonium, so that the cross-wall together with the tube make up a funnel-like protrusion, which, later, may frequently be seen with narrowed open end touching the oospore. Similar fertilization takes place in instances where, on conversion into an antheridium, the portion of hypha concerned undergoes slight external modification by becoming perceptibly distended at the end immediately adjacent to the oogonium (Fig. 7, B, a; L, a; Fig. 8, O, d; P, b). Further modification in antheridial shape, through the production of a lateral outgrowth arising always from a position close to the oogonium, permits intrusion of the fertilization tube through the spherical wall of the female organ. Depending on the measure of modification the lateral outgrowth may be of small volume in comparison with the cylindrical portion (Fig. 8, C, d; J, b); or, again, in instances where the cylindrical component is reduced to a very short segment, it may provide the main bulk of the antheridium (Fig. 7, D, b; J; Fig. 8, B, e; N, a). The latter condition approaches that represented in the frequent instances where the outgrowth is cut off by a basal septum to form by itself a male organ approximately sessile on the oogonial hypha (Fig. 7, B, b; D, a; E, e, d; H, a, b; I, f, g, h; Fig. 8, A, d; F; H, a, b; N, b; P, a). Often, especially when the outgrowth attains a somewhat greater length, the septum is laid down at an appreciable distance from the parent filament, with the result that the delimited male organ is borne terminally on a stalk of variable length arising, of course, always from a position very close to the oogonium (Fig. 7, C, c, d; G, a, b; H, e; I, i; Fig. 8, A, f, g; B, h; E; K, b).

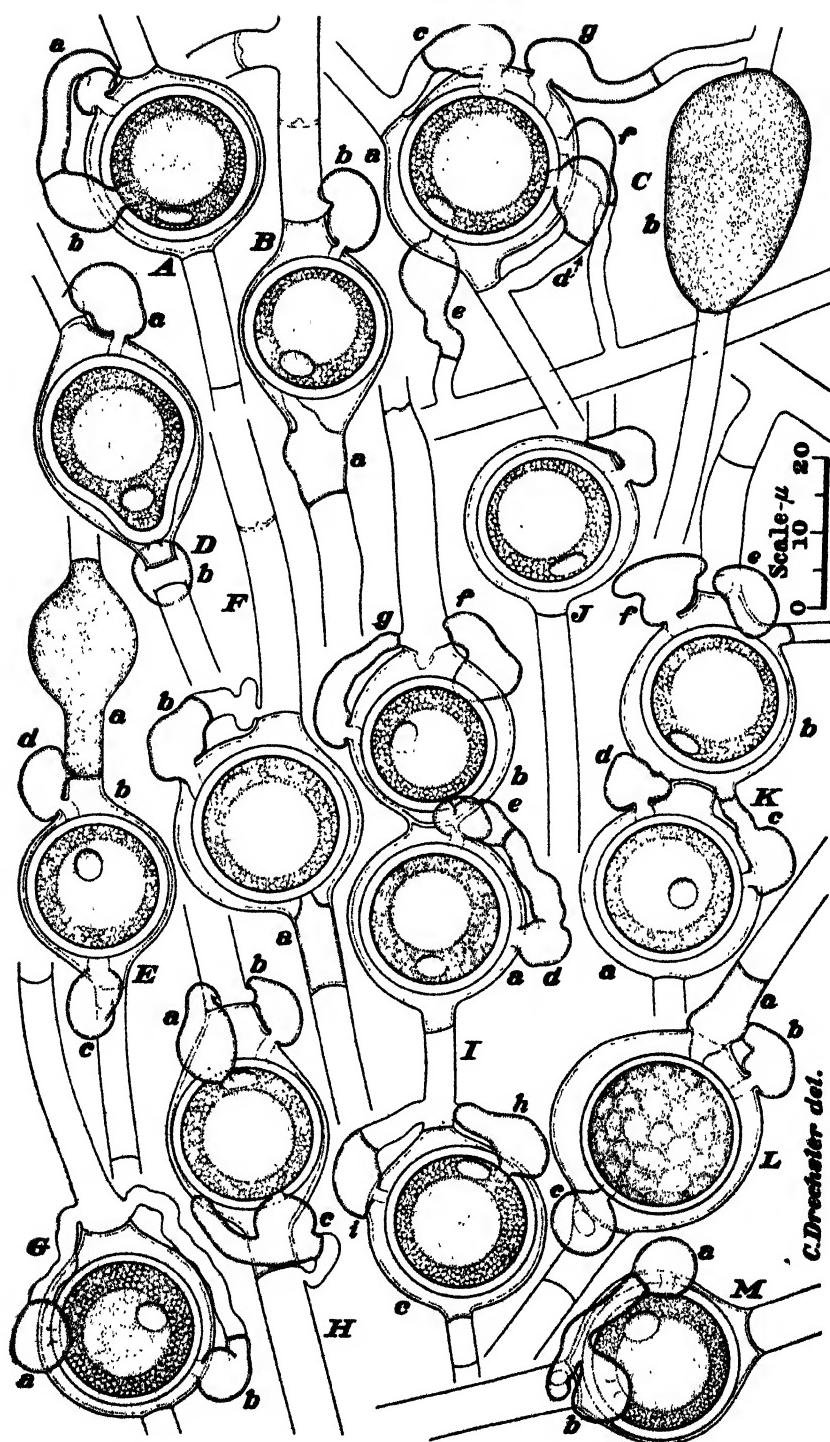


FIG. 7. Sexual reproductive apparatus of the type strain of *Pythium paroecandrum* isolated from field garlic, drawn with aid of a camera lucida;  $\times 1000$  throughout.

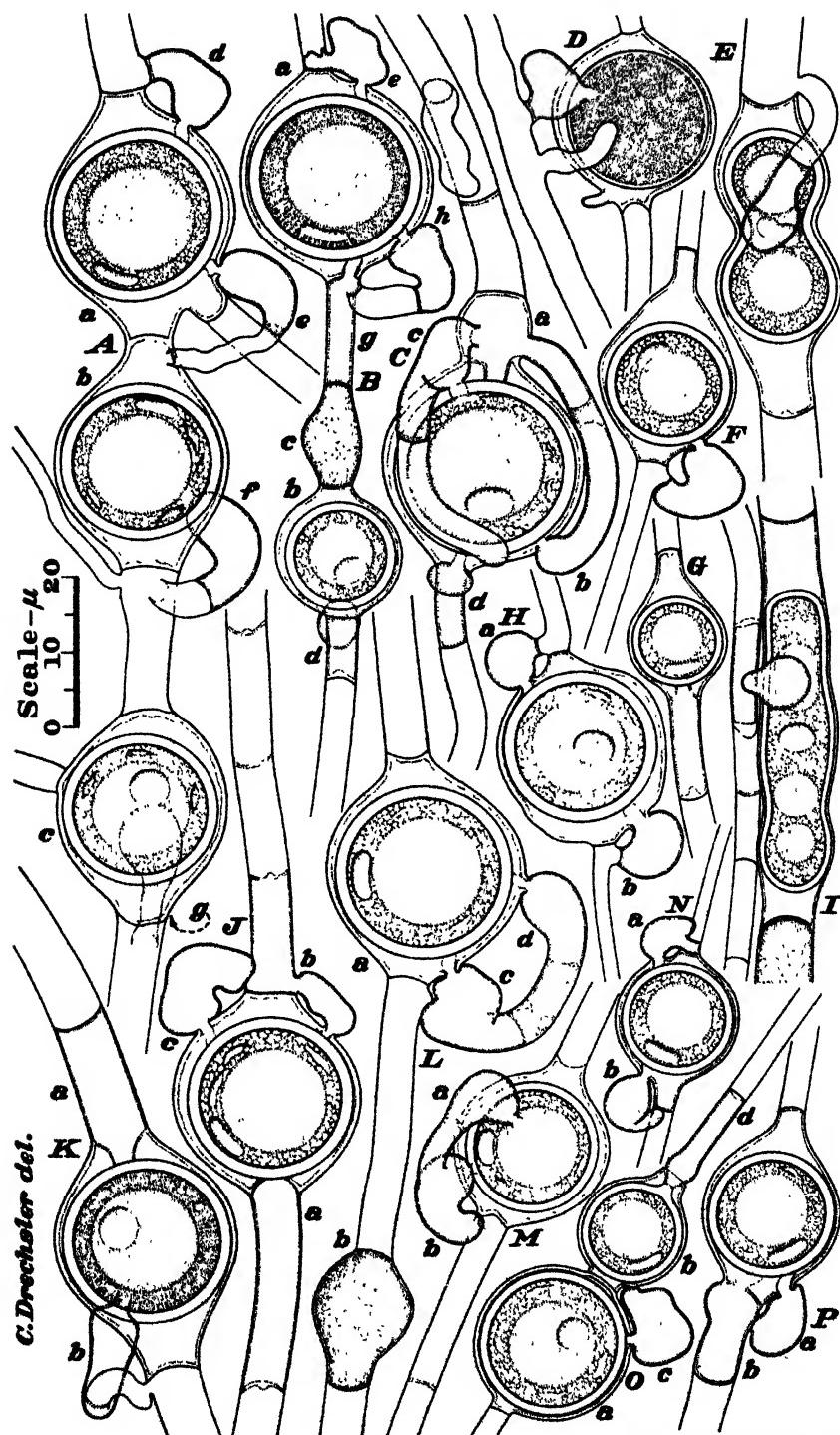


FIG. 8. Sexual reproductive apparatus of a strain of *Pythium paroecandrum* isolated from bloodroot, drawn with aid of a camera lucida;  $\times 1000$  throughout.

Antheridia thus differing considerably in manner of origin are found variously associated in many units of sexual apparatus. Not infrequently a lateral outgrowth, delimited at its base by a cross-wall and divided by a median septum, comes to constitute 2 antheridia arranged in series; the basal portion, sometimes with a pouch-like excrescence of its own, serving as a male organ no less than the distal portion (Fig. 7, A, a, b; I, d, e; M, a, b; Fig. 8, M, a, b). Likewise an antheridium composed wholly or in large part of a segment of hypha adjacent to the oogonium, may be contiguous with a sessile antheridium borne laterally on it (Fig. 7, L, a, b; Fig. 8, J, b, c; P, a, b), or may lack such contiguity only because the lateral antheridium is provided with a stalk (Fig. 8, B, g, h). Occasionally 2 fertilization tubes may be intruded into an oogonium from a more or less rangy antheridial system only rather dubiously divided internally by a protoplasmic plug (Fig. 8, C, a, b; L, c, d). Now and then, too, a sessile lateral antheridium may bear distally an empty sterile hyphal prolongation (Fig. 8, C, c).

After their fertilization the oogonia of *Pythium paraecandrum* show the internal changes familiar in many congeneric species. During the earlier stages in the development of the oospore its contents appear aggregated into somewhat irregular, sizeable lumps of nearly homogeneous consistency (Fig. 7, L; Fig. 8, D). Reserve material of completely homogeneous consistency soon begins to accumulate in a globule at the center of the massed lumps. As the reserve globule increases in size the protoplasmic lumps in the narrowing peripheral layer show indications of radial orientation (Fig. 7, I, a; Fig. 8, B, a; K) before becoming resolved into finer granules. At early maturity the oospore reveals unitary structure, its relatively large single reserve globule being surrounded by a rather narrow parietal granular layer in which is embedded a single refringent body, occasionally sub-spherical in shape, but more usually rather strongly flattened (Fig. 7, A; B; C, a; D; E, b; F; G; H; I, b, c; K, a, b; M; Fig. 8, A, a, b; B, b; C; F; G; H; J; L, a; M; N; O, a, b; P).

In cultures showing abundant sexual reproduction 2 oogonia may often be found immediately adjoining each other, their chambers separated only by a delimiting cross-wall (Fig. 7, I, a, b; K, a, b; Fig. 8, A, a, b; O, a, b). Frequently in instances of such contiguity one of the female organs is found supplied with antheridia (Fig. 7, I, d, e; K, c, d; Fig. 8, A, e; O, c) coming from its adjacent neighbor, which, therefore, on casual inspection presents the appearance of a bisexual structure. On more careful examination the presumption of bisexuality is not sustained. Evidently the antheridia of apparently anomalous origin arise, individually, like other male organs in the species, from an unmodified portion of hypha adjoining the oogonium they are destined to fertilize, and come to have their curious positional relationship only when the portion of hypha in question is utilized directly in the production of a second oogonium contiguous with the first. Should the portion of hypha be utilized instead in the development of a contiguous sporangium, any antheridium it may have put forth will seem to have arisen from the asexual reproductive body (Fig. 7, E, d).

In *Pythium paroecandrum*, as in many related fungi, oogonia and oospores departing markedly from a spherical shape are occasionally produced. Mycelium that has become largely exhausted in reproduction seems more inclined than young mycelium to afford development of malformed oogonia, wherein may be developed cylindrical oospores measuring perhaps 40  $\mu$  in length and 10  $\mu$  in width (Fig. 8, I), or, again, oospores of shapes suggestive of a dumb-bell (Fig. 8, E). Such oospores are often extensively adnate to the oogonial wall, and internally may reveal 2 (Fig. 8, E) or 3 (Fig. 8, I) reserve globules. The partly multiplicate internal structure represented here is manifestly referable to spatial exigencies, and is therefore not to be confused with the distinctive multiplicate structure characteristic of the oospores of *P. helicoides* Drechsl. and its allies.

Oogonia and oospores of such atypical form were excluded from consideration in the 200 measurements on which were based the statements given in the diagnosis relevant to the main dimensions of the fungus. Apart from this meager discrimination the measurements were made on units of sexual apparatus selected at random in maize-meal-agar cultures of the strain originating from field garlic,—each of the cultures used showing very abundant sexual reproduction with virtually no degeneration. The 200 mature oogonia gave values for diameter that when expressed to the nearest micron were distributed as follows: 11  $\mu$ , 1; 15  $\mu$ , 1; 16  $\mu$ , 1; 17  $\mu$ , 1; 18  $\mu$ , 3; 19  $\mu$ , 11; 20  $\mu$ , 33; 21  $\mu$ , 52; 22  $\mu$ , 51; 23  $\mu$ , 27; 24  $\mu$ , 15; 25  $\mu$ , 3; 27  $\mu$ , 1; and the 200 oospores of correct unitary internal structure contained within the oogonia gave values for diameter that when expressed to the nearest micron showed the following distribution: 10  $\mu$ , 1; 13  $\mu$ , 1; 14  $\mu$ , 1; 15  $\mu$ , 1; 16  $\mu$ , 8; 17  $\mu$ , 39; 18  $\mu$ , 71; 19  $\mu$ , 54; 20  $\mu$ , 18; 21  $\mu$ , 5; 22  $\mu$ , 1.

While in its asexual reproductive phase *Pythium paroecandrum* is closely similar to *P. debaryanum*, *P. irregularis* and *P. mammillatum*, it differs markedly from these species in its sexual phase; for, as has been noted, wherever its oogonia are fertilized by antheridia coming from the same filament, the antheridia in question are never borne, as usually in the 3 congeneric forms mentioned, on branches arising some distance from the female organ, but either consist in whole or in part of an adjacent portion of filament, or constitute the whole or a part of a process arising laterally from the axial filament in immediate proximity to the female organ. To be sure, the antithesis between *P. paroecandrum* and *P. debaryanum* with regard to origin of antheridia in monoclinous sexual apparatus is not a complete one, as the latter species also reveals, though only rather sparingly, male organs arising in proximate relationship to the oogonia. The same partial antithesis has been set forth earlier (6, 11) in distinguishing *P. ultimum* from *P. debaryanum*. Certainly, in their antheridial relationships, *P. paroecandrum* and *P. ultimum* present a striking parallelism. However, the mature oospore of *P. paroecandrum* differs rather markedly from that of *P. ultimum* in the greater size, proportionally, of its central reserve globule, in the correspondingly lesser thickness of its parietal granular

layer, and in the frequently much flattened shape of its refringent body. Further, of course, *P. paroecandrum* like *P. debaryanum*, *P. irregularare* and *P. mammillatum*, is separated from *P. ultimum* by its ready production of zoospores.

Before *Pythium paroecandrum* was described as new its morphological features were considered in comparison more especially with the morphological details given by Butler (3) in the original account of his *P. rostratum*. Although the measurements for diameter of zoosporangium given by Butler somewhat exceed those of my fungus, the difference could not be regarded as sufficient for the separation of 2 species. Even less disparity was evident with respect to size of oogonium. If the antheridium of *P. rostratum*, described as being generally single, and as consisting often of a short hyphal segment adjacent to the oogonium, or of such a segment together with a short lateral process arising therefrom, fails to embody the whole range of variability revealed by antheridia in monoclinous sexual apparatus of my fungus, it yet could be recognized as unmistakably embodying an important part of that range. The chief difference impelling separation was much the same as that on which Butler based the separation of his species from *P. debaryanum*. For, in *P. paroecandrum*, as in *P. debaryanum*, and also as in *P. ultimum*, during a long period assimilated to *P. debaryanum*, the oospore is considerably smaller than the oogonium, and is, therefore, loosely contained within the oogonial chamber; whereas, in *P. rostratum*, the oospore completely or nearly completely fills the oogonium. The distinction appears all the more valid from the circumstance that Butler studied his fungus in water cultures, where oospores of many species of *Pythium* tend to become smaller in proportion to the oogonium than on firm agar substrata. According to Butler, moreover, the tube of discharge in the sporangium of *P. rostratum* is characteristically thickened about midway in its length. By way of contrast the evacuation tube produced by the sporangium of *P. paroecandrum* does not show consistently any distinctive localized modification; its tendency toward occasional crookedness and toward distal widening being shared by the homologous elements of many related species.

From de Bary's publications (1, 2) on his *Pythium proliferum* and *P. ferox*, it seems clear that these fungi produce antheridia in proximate relationship to the oogonium. However, the terminal proliferous sporangium, characteristic of *P. proliferum* and found presumptively also in *P. ferox*, represents a type of asexual reproductive body differing rather widely from the more commonplace, usually intercalary, nonproliferous sporangium of the present form.

In *Pythium pulchrum*, which, according to its original description by von Minden (18), also produces antheridia adjacent to oogonia, the oogonia and oospores have average diameters of 28  $\mu$  and 24  $\mu$  respectively, and are, therefore, considerably larger than the corresponding structures of *P. paroecandrum*. The clustered basipetal development of sporangia, figured

by von Minden, has never been observed in irrigated preparation of my fungus. Several cultures that I have isolated from aquatic material and that show satisfactory agreement with the description of *P. pulchrum*, are assuredly alien to *P. paroecandrum*.

Höhnk (14), in his original account of *Pythium epigynum*, dealt with a form wherein, again, mostly intercalary, subspherical zoosporangia comparable in size to the zoosporangia of *P. paroecandrum* are associated with oogonia that only slightly exceed those of my fungus in average diameter and that apparently are regularly fertilized by 1 or 2 antheridia consisting of somewhat swollen adjacent hyphal segments. From the discussion and illustrations given by him it is not apparent that antheridia individually consisting in whole or part of a lateral outgrowth arising in immediate proximity to the oogonium were ever observed, or that fertilization tubes ever entered the oogonial chamber except by penetration of the delimiting septum. The oospores of *P. epigynum*, judging from their range in measurements of diameter, 14 to 22  $\mu$ , mostly 18  $\mu$ , appear closely similar in size to those of *P. paroecandrum*, and, incidentally, would seem to have been more plausibly described in Höhnk's arresting phrase "oogone not filling" than in the deduction he drew from an overlapping of plotted curves representing values for diameters of oogonium and oospore. The rather meager dimensional overlap suggests not so much that some oospores fill the oogonia wherein they are produced, as that some of the larger oospores produced by the species, if they could be transferred, would fill some of the smaller oogonia produced by the species, and would, indeed, more than fill other still smaller oogonia produced by it.

Matthews (17), in illustrating *Pythium pulchrum*, gives figures among which some show much resemblance to Höhnk's figures of *P. epigynum* with regard to position and origin of antheridia. If the magnifications indicated for Matthews' figures are correct, the oogonia and oospores drawn by her would appear, besides, to have had dimensions approximately equal to the homologous dimensions of *P. epigynum*, despite an implication in her account that the drawings were prepared from a culture producing oogonia even somewhat larger than the oogonia of von Minden's fairly robust fungus. However, the catenate arrangement of zoosporangia set forth by Matthews received no mention in Höhnk's account. It is not apparent that either of the 2 fungi studied by these authors had a range of variations in antheridial relationship comparable with that revealed by *P. paroecandrum*.

In describing his *Pythium piperinum* as a new species causing root rot of pan, *Piper betle* L., and of pipri, *P. longum* L., Dastur (4) mentioned that its antheridia may consist of a branch from the oogonial hypha, or may develop directly from this hypha. All 3 of Dastur's drawings of sexual apparatus show plural antheridia in positions near an attachment of the oogonium to the supporting filament, suggesting that the male organs were derived from lateral outgrowths put forth by the filament close to the oogonium. In their measurements the oogonia and oospores of *P. piperinum*

appear only slightly smaller than those of *P. paroecandrum*. However, the zoospores of the Indian fungus, measuring only 3.4 to 5.1  $\mu$  would seem not only smaller than the swarm spores of *P. paroecandrum*, but smaller than any normal swarm spores I have seen produced by any species of *Pythium*.

#### SUMMARY

Many zoosporangia of *Pythium dissotocum* consist of completely undifferentiated filaments, while others include a number of somewhat distended lateral branches. They yield enormous numbers of zoospores, which often are much given to iterant swarming. In the sexual apparatus of the species are revealed some antheridial relationships familiarly exemplified in *P. debaryanum* and *P. ultimum*.

*Pythium peritium* displays swollen elements more abundantly in its zoosporangia than *P. dissotocum*. Its oogonium is extensively and closely inwrapped by a branching antheridial filament, much like the oogonia of *P. scleroteichum* and various terrestrial species of *Aphanomyces*.

*Pythium paroecandrum* produces subspherical zoosporangia like those of *P. debaryanum*. As is implied in the specific epithet, a term compounded of 2 words meaning "neighbor" and "man" respectively, its antheridia often arise in close proximity to the oogonium. Thus, in arrangement of sexual apparatus, the species greatly resembles *P. ultimum*, although the oospore with its relatively large reserve globule has an internal organization more suggestive of *P. debaryanum*.

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## VOLATILE FUNGICIDES, BENZOL AND RELATED COMPOUNDS, AND THE PRINCIPLES INVOLVED IN THEIR USE<sup>1, 2</sup>

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### INTRODUCTION

For several years the use of volatile fungicides to control downy mildew of tobacco has been given consideration both in Australia and in the United States. The information gained has been largely assembled in previous reports (1, 2, 3, 4, 6, 8, 9, 10, 13). The present paper comprises two portions. The first deals with experiments involving the application of benzol at intervals of more than one night between successive treatments, and with experimentation on types of evaporators. The second details experiments concerning the mode of action of fumigant fungicides that are basic to the subsequent discussion of the principles involved in the use of volatile compounds. This portion is timely because volatile materials, aside from benzol, are indicated as useful not only in control of tobacco downy mildew but of various other plant diseases.

### APPLICATION OF BENZOL AT REGULAR INTERVALS OF MORE THAN ONE NIGHT

In our previous reports (7, 9, 13) involving the use of benzol, each successive night, as a means for the prevention and control of tobacco downy mildew in seed beds, attention was called to the fact that satisfactory control can be obtained when the interval between successive applications is longer than one night (13). The possibility of the more efficient use of materials

<sup>1</sup> Cooperative investigation conducted by the Virginia Agricultural Experiment Station and Duke University.

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and labor in connection with fumigation of seed beds with benzol made it desirable to gain additional information as to the efficacy of intermittent treatments at these longer intervals. During the season of 1939, therefore, experiments involving the application of benzol regularly at longer intervals were conducted near McDonald and Oxford, North Carolina and Chatham, Virginia. These locations are representative of the area devoted to the growing of flue-cured tobacco. The applications at each of these localities were made according to a predetermined schedule. In addition, near Chatham, benzol was applied when indicated by best judgment.

The seed beds used were tightly constructed of boards. They were divided into compartments with an area of approximately 8 sq. yd., most of them having a width of 6 ft. Large beds, 10 to 12 ft. wide, were employed in a few cases. The covers were unbleached cotton sheeting of the following specifications: threads per inch, warp 64, woof 64, sq. yd. to weigh 1 pound, 2.7. The covers were thoroughly wetted after fastening them tightly in position. The surface area of the pans used for evaporating the benzol was 100 sq. in. The intervals between successive applications in the different series were 1, 3, and 4 nights, respectively. The applications were continued for approximately a month. The amounts of benzol applied were 37.5, 50, 62.5, 100 or 200 ml. per sq. yd. of seed-bed area per application. The areas involved were 40 sq. yd. in 5 compartments treated every alternate night, 382 sq. yd. in 7 compartments treated every third night, and 24 sq. yd. in 8 compartments treated every fourth night.

The outstanding conclusion from these experiments is that benzol need not be applied every night in order successfully to control downy mildew. It should be indicated that a slight amount of sporulation had taken place in all cases, prior to making the first application, and that downy mildew was completely checked in each of the series, except one, by a single application of benzol. In this case sporulation was rather abundant throughout the bed at the initiation of treatment, and benzol was applied on two successive nights to make certain that the disease was checked.

A few flecks with necrotic centers appeared on the older leaves of some of the treated seedlings. Careful daily examinations of each bed, for the occurrence of sporulation, revealed only one or two leaves bearing sporangia in any of the beds throughout the entire period of treatments. None of the seedlings in the treated beds succumbed to downy mildew, whereas all plants in nontreated control beds were attacked, and from 25 to 75 per cent of them were killed. The appearance of one such bed, part of which was treated every fourth night and the other part, nontreated, as a control, is shown in figure 1, A and B. The treated seedlings (Fig. 1, A) were essentially of sufficient size to transplant, while the few surviving seedlings in the nontreated portion (Fig. 1, B) had just begun to recover.

The Mine Safety Appliance Combustible Gas Indicator was again used (13) to measure the benzol-vapor concentration in the atmosphere of certain beds. Attention is called to the fact established in previous investigations

(13) that concentrations of benzol vapor in excess of 0.4 volume per cent are lethal to the downy mildew pathogen. The concentrations obtained during the greater portion of the nights in these beds were fungicidal with each of the amounts of benzol employed.

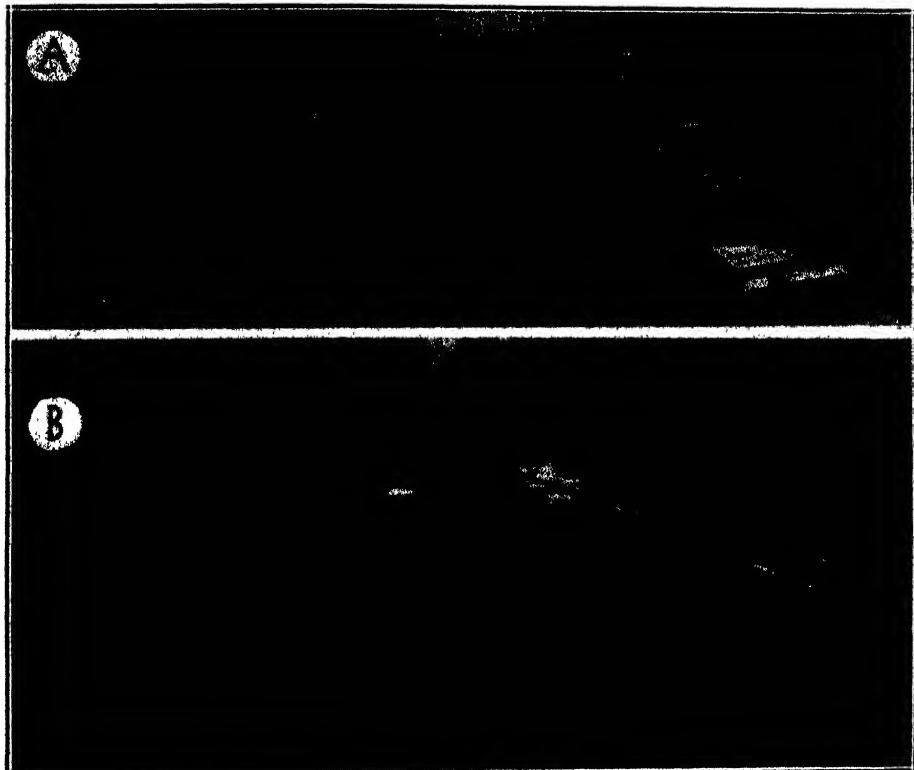


FIG. 1. A. Portion of seed bed to which benzol was applied regularly at intervals of 4 nights. (This also appears in the background of B.) B. Portion untreated. Photographs taken on same date as A.

The maintenance of fungicidal concentrations was possible by virtue of two factors, (1) moisture on the covers and foliage, and (2) amount of benzol applied. It should be emphasized that moisture on the covers and foliage, as previously stated (6, 13), is the most essential condition to the effective use of benzol in seed beds. The amount of benzol applied was in most cases in excess of what would evaporate overnight. Use of the larger amounts was followed by slight leaf-tip injury, accompanied by more or less yellowing or blanching of the older foliage and, in some cases, by dwarfing of the seedlings (Fig. 2). Under our conditions approximately 35 ml. per sq. yd. of seed-bed area appears to be the maximum quantity allowable.

#### APPLICATION OF BENZOL AT IRREGULAR INTERVALS

As a result of successful prevention and control of downy mildew of tobacco by applying benzol according to a fixed schedule at the longer

intervals between successive applications, it seemed logical to apply the fungicide at irregular intervals, as indicated by judgment. Time of application of benzol was based on the following factors: (1) comprehension and appreciation of influence of temperature and rainfall on severity of the disease; (2) daily observations on progress of downy mildew not only in experimental but in near-by seed beds; (3) observations made each morning on abundance of sporulation; and (4) knowledge of influence of weather on dissemination and germination of sporangia.

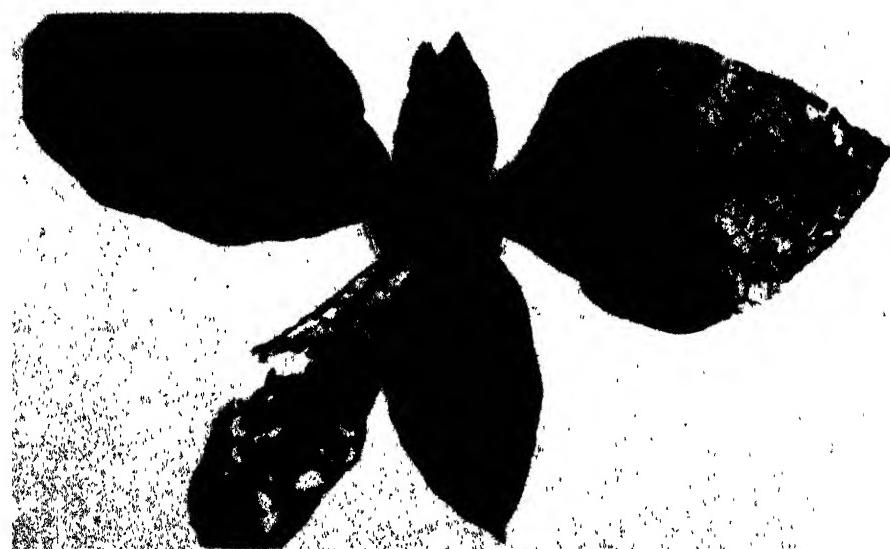


FIG. 2. Blanching of intercostal tissues characteristic of benzol injury to tobacco seedlings.

Treatment of both experimental and growers' seed beds in the manner described above was attempted near Chatham, Va. Applications of the fungicide were made first immediately after downy mildew appeared in the beds. Four to 6 additional applications so controlled the disease that the foliage was only slightly flecked.

#### DAYTIME APPLICATION OF BENZOL

In the hope of being able to shorten the period of treatment, several series of attempts were made to control tobacco downy mildew by applying benzol on clear warm days. Amounts ranging from 25 to 150 ml. per sq. yd. of seed bed area, were evaporated by means of pans, wick devices, and saturated compacted "cotton balls." The periods of treatment varied from 1 to 4 hours. Seed-bed covers were kept wet during the treatments. From these experiments it appears that the heavier applications result in severe seedling injury. There was evident no eradicate action from applications of 25 to 50 ml. per sq. yd., even when the period of treatment extended for

3 hours on each of 2 successive daily applications. Daytime applications promise no success because of the difficulty of keeping the covers sufficiently wet to confine the benzol vapors to the seed beds, of keeping a film of moisture on the foliage, and the ineffectiveness of short-duration treatments.

#### OTHER MATERIALS AND METHODS OF TREATMENT

Several preliminary tests of other volatile materials have been made to determine their value as downy-mildew fungicides. These materials included S. T. 28, prepared by The Shell Oil Co.; aniline, phenol, aniline hydrochloride, paratoluidine, and pyridine. The results with each compound, except paratoluidine, are inconclusive. Aniline and paratoluidine caused severe seedling injury, and the latter was non-fungicidal in our tests.

In a previous report it was pointed out that benzol vapor rather quickly enters into aqueous solution and that water constitutes the vehicle through which benzol becomes fungicidally active. It therefore seemed logical to test dilute aqueous solutions of benzol as sprays. These tests failed to demonstrate the practical value of sprayed benzol solutions as a downy-mildew fungicide, although their application proved beneficial. The limited fungicidal efficiency of benzol in aqueous solution derives from the large amounts of water required and the necessity of frequent applications. Its use as a spray for control of other diseases remains wholly unexplored; and the possible value of dilute solutions in soil sterilization, seems worthy of study.

Dilute aqueous solutions of aniline, aniline hydrochloride, and pyridine, sprinkled upon the seedlings, were markedly injurious, even in concentrations that gave no evidence of being fungicidal. The results with aqueous solutions of phenol indicated that this compound should be given further study as a means of controlling downy mildew.

#### TYPES OF EVAPORATORS

Appliances, previously used as evaporators for benzol, include pans, troughs, perforated tubes, and devices with wicks. Although all are not equally good, it is possible to secure with each type satisfactory control of tobacco downy mildew. Furthermore, it has been found that seed beds not to exceed 6 feet in width facilitate installation and care during operation of any of these types of evaporators. Also such beds obviate the necessity of trampling in them while applying the fungicide.

An ideal evaporator should be inexpensive, easy to install and replenish, and durable. It also should deflect rain and permit vaporization to proceed at a uniform rate. Efforts to devise such an evaporator have been only partly successful and have led to experimentation with "cotton balls." A cotton ball consists of 30 g. of compacted non-absorbent cotton, covered with cloth. These balls are then dipped in benzol and suspended from properly spaced stakes within the beds. One cotton ball for each 4 sq. yd. of seed bed has been found sufficient. Some canopy should be provided to deflect the rain.

## GENERAL CONSIDERATIONS BEARING ON FUMIGATION WITH BENZOL

Although, in 1939, entirely satisfactory control of downy mildew was secured by application of benzol at intervals of 1 to 4 nights between successive applications in 3 widely separated localities, there remains some basis for doubt whether equally satisfactory results could be secured every season. It is probable that the disease might not yield to treatment in localities where downy mildew yearly destroys nearly all of the seedlings unless the treatments are made every night. Such appears to have been the case in the experiments of Allan, Hill, and Angell (1). They report successful control in certain tobacco-growing areas in New South Wales and Queensland, if treatments are made on alternate nights, but failure with similar treatments in other areas. From their account it is not clear that their mode of application could be considered as effective as that described here. Our results indicate that benzol acts as an eradicant fungicide, provided that (1) the proper amount of benzol be applied, (2) the rate of evaporation as modified by size of the evaporators and by temperature, be favorable, and (3) the tightness and moisture conditions of the bed favor retention of the enclosed vapors.

Since the outbreak of downy mildew in 1931, this disease was especially destructive in the United States in 1932 and 1937, and only moderately

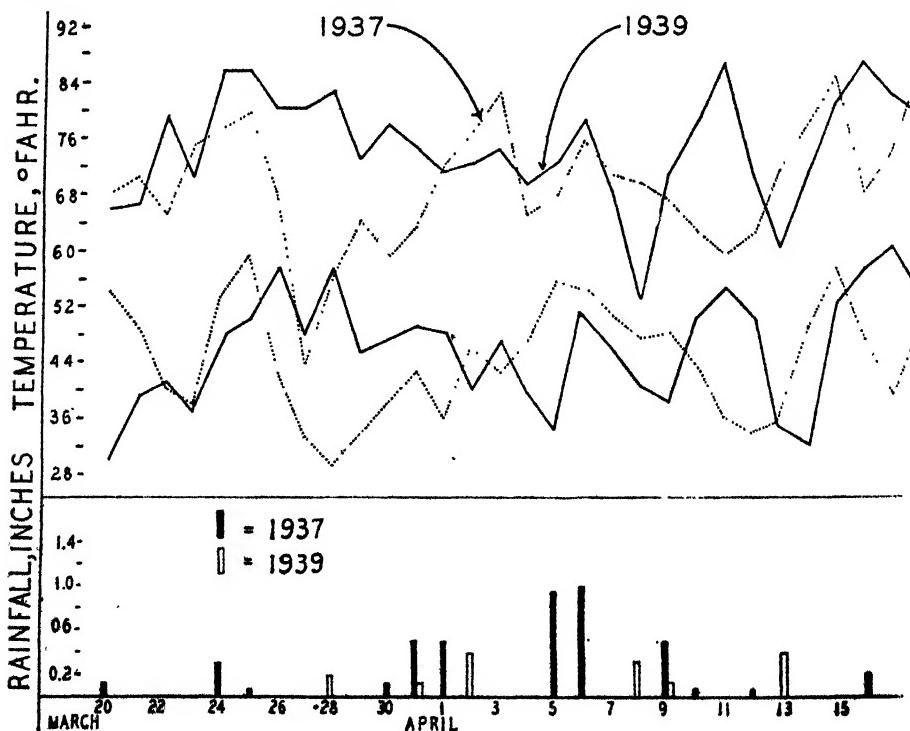


FIG. 3. Comparison of weather conditions at McDonald, N. C., from March 20 to April 16, 1937 and 1939. In 1937, 16 clear days, 12 partly cloudy or cloudy, and rainfall 4.1 inches. In 1939, 20 clear days, 8 partly cloudy or cloudy, and rainfall 1.35 inches.

severe in the remaining years. There is evidence (5) that differences in weather conditions may account for seasonal differences in destructiveness of the disease. This point is illustrated if weather conditions in 1937 and 1939, seasons in which the severity of downy mildew was strikingly different, are contrasted. Weather data for the critical 28-day periods of these two seasons, near McDonald, N. C., are presented in figure 3. It is here apparent that the season of 1937 was considerably colder than that of 1939. Almost 3 times as much rain fell in the former period as in the latter. Moreover, the proportion of cloudy and partly cloudy days was considerably greater in the former season. Whether, under such severe conditions, it is possible to control downy mildew by applying benzol at intervals greater than one night will require further field experimentation under weather conditions approximating those of the 1937 season.

#### EFFECT OF VOLATILE COMPOUNDS ON TISSUES OF TOBACCO SEEDLINGS AND ON SPORES OF PATHOGENS

Experiments on the effect of volatile compounds on the tissues of tobacco seedlings and on the spores of the pathogen will be detailed before entering into a discussion of the principles that are basic to treatment with volatile fungicides. This procedure provides evidence bearing on the mechanism of operation of toxic vapors.

#### The Toxic Action of Benzol and Paradichlorobenzene on Tobacco Seedlings

Tobacco seedlings injured by fumigation with benzol or with paradichlorobenzene have quite the same appearance. It may be recalled that injury from benzol (Fig. 2) has been briefly described (9). Slight toxicity from paradichlorobenzene induces yellowing of the foliage and the appearance of lesions near the leaf tips. If cloudy weather follows injury of the plants, these lesions become pale brown; if clear days occur they are bleached until nearly white. This observation is based upon experiments in which portions of injured leaves were screened from direct sunlight by black paper.

In a previous report (9) consideration was given to the mode of action of benzol. It was postulated that benzol modifies the permeability of the plasma membranes by reacting especially with the lipoidal constituents of these membranes. This hypothesis was based not upon experiments designed to elucidate the mode of action but upon a rationale from the findings of those who have studied the toxicity of benzol to animals and upon the probability that the mechanism of toxic action to animal and to plant cells can be expected to accord in certain features.

In efforts to determine whether permeability of the leaf cells of tobacco seedlings is changed as the result of exposure to vapors of benzol or paradichlorobenzene, electrical conductance tests were employed. As plant physiologists well know, injured tissues exhibit increased permeability and the electrical conductivity of their fluid extracts is increased. In conse-

quence, electrical-conductance measurements have been accepted as a standard method for measuring the changed electrolyte concentration. Accordingly, leaf tissues from seedlings injured by exposure to vapors were placed in distilled water, and the samples were then put in a cold room to permit exosmosis. Tissues from normal leaves, similarly extracted, served as controls. After an appropriate interval, measurements were made by means of a conductivity apparatus equipped with audio-amplifiers and head phones. The results of representative measurements are recorded in table 1.

TABLE 1.—*Specific conductivities ( $\times 10^6$ , at 30° C.), expressed in reciprocal ohms, of water extract from leaves of tobacco seedlings. An interval of eight hours was allowed for exosmosis at 3° C.*

Series	Treatment of seedlings	Specific conductivity
1	Exposed over night to toxic conc. of para-dichlorobenzene	404
	Nontreated control	135
2	Seedlings very severely injured by exposure to vapor of paradichlorobenzene	517
	Injured severely by treatment with benzol	1213
	Nontreated control	186
3	Exposed to vapors of paradichlorobenzene	641
	Leaves boiled in water	962
	Nontreated control	135
4	Seedlings exposed for 36 hours at 13° C. to atmosphere saturated with para-dichlorobenzene	800
	Seedlings treated for 12 hours at 30° C. with paradichlorobenzene	780
	Nontreated controls	112

The most apparent conclusion deducible from these data is that leaf tissues injured by vapors of benzol or paradichlorobenzene are less able to prevent the loss by exosmosis of electrolytes. It must follow, therefore, that these volatile compounds increase the permeability of the plasma membranes. The degree of its modification apparently is directly correlated with the amount of injury or the extent to which the leaf tissues or entire seedlings collapse.

#### Germination of Sporangia of *Peronospora tabacina* in Solutions of Volatile Substances

A voluminous literature exists on methods of testing the fungicidal effects of chemicals. Wilcoxon and McCallan (12) state that studies on toxicity of fungicides are of 2 types: those in which quantitative measurements are made of some property of the individual, as, for example, length of germ tube or diameter of colonies; and those in which the individuals are divided into 2 categories, i.e., percentage of germinated and nongerminated spores. In toxicity studies of the latter type it is well known that

each spore has its own particular lethal dose, a factor that makes impossible the procurement of a definite endpoint. Instead, it is found that a curvilinear relationship exists when the distribution of individual lethal doses is plotted against the logarithm of the concentration.

The techniques employed in tests of toxicity of non-volatile fungicidal substances have largely become standardized. This is not the case, however, at least to the same extent, with volatile fungicides, since the *sine qua non* of such tests is the use of closed germination chambers to maintain continuously the desired concentration of the chosen chemical. The chemical to be tested, moreover, should not be soluble in the material used to seal the germination chambers.

In his study of volatile fungicides Tomkins (11) used ground-glass-top jars. The molds were grown on agar solidified in the tops and inverted above the solutions of the volatile materials. Oserkowsky (8) placed vials containing saturated solutions in jars and surrounded the bases of the vials with agar. He also immersed the sclerotia of *Sclerotium rolfsii* in saturated solutions of benzol and a number of other compounds. In our experiments aqueous stock solutions, saturated at 30° C. with benzol, paradichlorobenzene, phenol, and aniline were prepared and kept in glass-stoppered bottles. Dilutions of each were made when it was desired to test fungicidal value. Hollow-ground slides, van Tieghem cells, and stoppered capillary tubes proved unsatisfactory and were discarded along with various substances used as seals. The germination chambers (Fig. 4) consisted of glass vials, 35 × 18 mm., with ground edges. These were filled almost to

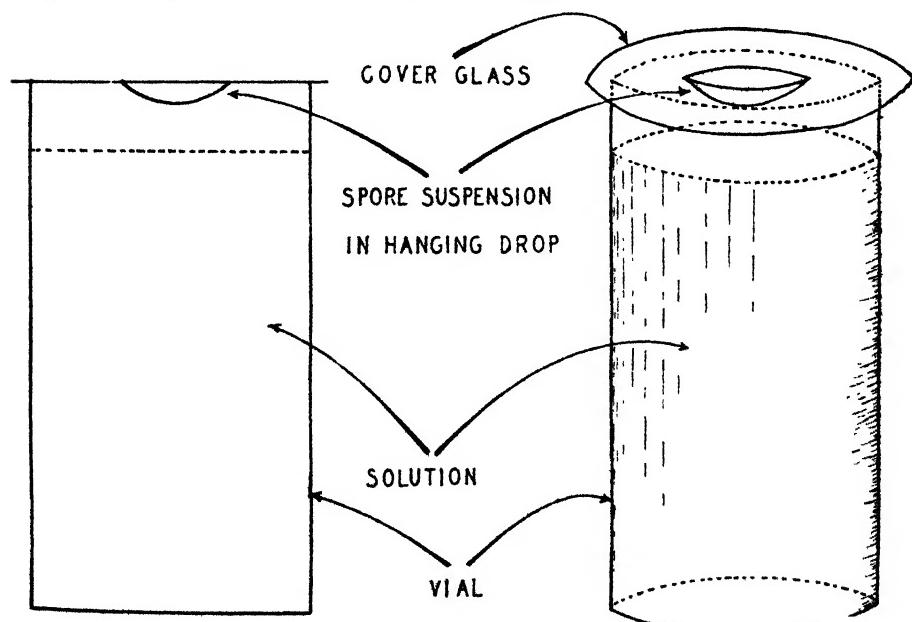


FIG. 4. Diagram of devices used in determining concentrations of volatile compounds that inhibit germination of sporangia of *Peronospora tabacina*.

the top with an appropriate dilution of the chemical to be tested. The top of each was closed with a cover glass bearing sporangia suspended in a hanging drop. A film of the test solution applied at the ground-glass edge of the vial made an effective seal, as shown by the fact that the hanging drops remained intact for 10 or more hours. Immediately after the hanging-drop suspensions had been made the germination chambers were placed in a chamber whose basal part contained water to provide them with an atmosphere of high relative humidity. Temperatures favorable for germination were then provided. The tests were started between 7 and 8 a.m. and examinations were made between 5 and 7 p.m.

Freshly formed sporangia were employed, an essential prerequisite made

TABLE 2.—*Germination of sporangia of Peronospora tabacina at 10° C. to 12° C. in aqueous solutions of benzol, paradichlorobenzene, phenol, and aniline*

Cone. of chemical	No. of tests	Results	Remarks
1/8 saturated benzol	8	No germination	Sporangia plasmolyzed
1/10 saturated benzol	3	No germination	Sporangia plasmolyzed
1/12 saturated benzol	2	No germination	Germ tubes mere protrusions
1/14 saturated benzol	6	Few sporangia germinated	Germ tubes bud-like
1/16 saturated benzol	12	In 8, few germinated; in 4, none	Germ tubes 2-4 times the length of the sporangia
1/18 saturated benzol	4	Germination abundant	Normal length of germ tubes
1/20 saturated benzol	2	Germination abundant	Normal length of germ tubes
1/24 saturated benzol	4	In 3, abundant; in 1, none	Normal length of germ tubes
1/32 saturated benzol	5	In 4, abundant; in 1, none	Normal length of germ tubes
3/4 saturated paradichlorobenzene	6	No germination	
5/8 saturated paradichlorobenzene	4	In 2, few germinated; in 2, none	Germ tubes the length of the sporangia
1/2 saturated paradichlorobenzene	12	In 8, few germinated; in 4, none	Germ tubes shorter than normal
3/8 saturated paradichlorobenzene	4	Abundant germination	Germ tubes normal length
1/4 saturated paradichlorobenzene	7	Abundant germination	Germ tubes normal length
1/600 saturated phenol	8	No germination	
1/650 saturated phenol	4	In 1, few germinated; in 3, none	Germ tubes short
1/700 saturated phenol	8	Meager amount of germination	Germ tubes short
1/750 saturated phenol	4	Many sporangia germinated	Germ tubes not of normal length
1/800 saturated phenol	2	Excellent germination	Germ tubes normal
1/50 saturated aniline	16	In 6, few germinated weakly; in 10, none	Germ tubes short
1/75 saturated aniline	12	In 6, only slight amount of germination	Germ tubes short
1/100 saturated aniline	7	Good to excellent germination	Germ tubes normal

possible by keeping a supply of infected tobacco seedlings in a box constructed to maintain temperature and moisture conditions favorable for sporulation. Control germination tests were made daily under conditions identical with that described above, except for absence of the fungicide.

A summary of the results of tests to determine the toxicity of certain volatile chemicals is contained in the following tabulation.

Apparently, concentrations of  $\frac{1}{4}$  saturated benzol,  $\frac{1}{2}$  saturated para-dichlorobenzene, 1/750 saturated phenol, and 1/75 saturated aniline closely approximate the minimal fungicidal concentrations of these substances for *Peronospora tabacina*. It should be said in explanation that the percentage germination of sporangia was not recorded, for it is known that they vary extremely in ability to germinate in water.

#### Implications from Sporulation Experiments

Observations agree that sporulation may not occur on infected tobacco seedlings until 6 or 7 days after treatment with benzol, a period corresponding to the length of the sporangial cycle. It was concluded, for this reason, that benzol is lethal to the mycelium within the leaves (9). Whether it actually is killed is unknown, although this would be easily ascertainable were it possible to grow the organism in test-tube cultures. It has been im-

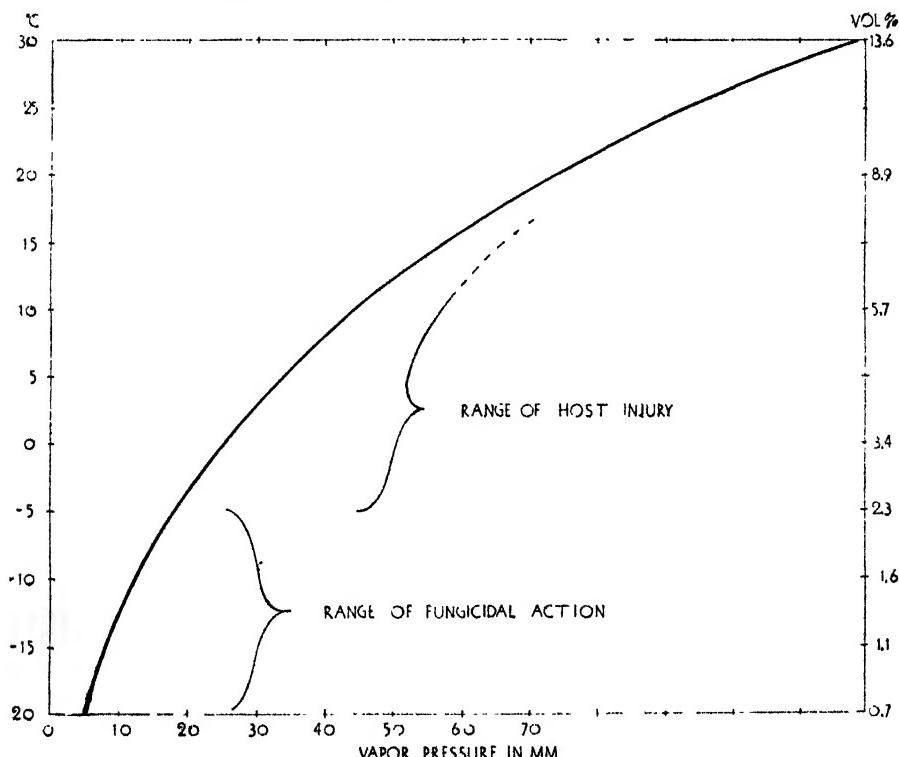


FIG. 5. Interrelationship of temperature, vapor, pressure, and volume-percentage concentration of benzol at saturation in air, showing the wide range in which benzol can be employed as a fungicide against downy mildew.

possible to determine with the microscope whether the fungus was alive or dead. No difference in appearance between the mycelium in leaves treated with benzol and that in non-treated leaves was detectable. Whether benzol produces a fungistatic or a fungicidal action is seemingly of little practical significance, since the net result is the control of the downy mildew disease. Protection may be complete if treatments are initiated prior to infection. If there is sporulation by the time of the first treatment a little flecking and necrosis may develop, but not enough to hinder transplanting.

### Principles Involved in the Use of Volatile Fungicides

Studies to determine the fungicidal value of volatile materials should be based upon an understanding of certain physico-chemical principles, especially the interdependence of temperature, vapor pressure, and volume percentage concentration of the vapors in air. In order to make it possible to visualize the relation of these factors to each other in the case of benzol, and their bearing on the use of this chemical to control tobacco downy mildew, available pertinent data are presented graphically in figure 5. This curve shows the partial pressure of benzol in a benzol-saturated atmosphere at the temperature indicated. In actual field practice saturation would never be obtained. The saturation pressures are such that fungicidal concentrations are possible at temperatures much lower than those that occur while seedlings are being grown. Since fungicidal action occurs within the range 0.4 to 0.5 volume-percentage vapor concentration, it is seen from figure 5 that saturation pressures of benzol vapor below -20° C. would, in fact, be effective. However, within the temperature range for growth of tobacco seedlings effective fungicidal concentrations occur at less than 0.1 saturation. Temperature, therefore, is never a limiting factor in the effectiveness of benzol as a downy-mildew fungicide.

It may be of interest to contrast certain related volatile compounds with benzol. For example, naphthalene, closely related structurally to benzol, might be anticipated to have a very similar specific toxic action. Laboratory and field tests, however, have shown that it does not successfully control tobacco downy mildew because of its inherently low volatility. Its vapor pressure at saturation, at 10° C., is 0.021 mm., whereas the corresponding value for benzol is 45.0 mm. On the other hand, if the toxic action be altered by changing the chemical structure, certain compounds with limited volatility may become valuable fungicides. Thus, if 2 chlorine atoms are introduced in the para positions into the benzene ring, both the volatility and the specific toxic action of the aromatic nucleus are altered. Although the compound formed, paradichlorobenzene, has a saturation pressure of only 0.23 mm. at 10° C., nevertheless, it is an effective fumigant.<sup>8</sup> Its saturation pressure (0.23 mm. at 10° C.) corresponds with a volume-percentage concentration of 0.031 per cent, being less than one-third of the volume-percentage concentration of benzol that was found necessary for

<sup>8</sup> From our unpublished data.

fungistatic action (13). When, however, the temperature is as low as 0° C., the saturation pressure of paradichlorobenzene drops to 0.089 mm. and the volume-percentage concentration of its vapors in air to 0.012 per cent. Since actual concentrations in seed beds would be far below saturation, the magnitude of the concentrations realizable at low temperatures might be so small as to make paradichlorobenzene an ineffective fungicide.

The above figures were obtained from laboratory experiments on infected seedlings subjected to a moving air current containing a constant vapor concentration of the fumigant. Under these conditions, equilibrium distribution of the fumigant between the vapor phase and the plant tissues could be approximated.

Monochlorobenzene, structurally intermediate between benzol and para-dichlorobenzene, has properties that are intermediate between the two compounds. Its vapor pressure at 10° C. is 4.9 mm., and sufficiently large concentrations of its vapors in air are obtainable to make it an effective fungicide, as has been demonstrated (7).

The mechanics of action of fumigant fungicides may be conceived to proceed as follows: When the vapors reach the leaf surface their absorption seems to take place mainly by solution in the surface film of moisture, although stomatal openings may serve the fumigant to some extent as inlets to the leaf tissues. This aqueous solution of the fumigant on the leaf surface, if sufficiently concentrated, is lethal to both sporangia and spores.

The widely differing results of treatment when the leaf surface is kept wet instead of dry during fumigation with benzol shows that solution of the fumigant plays an important part in determining its effectiveness. Although the volatile substances herein employed are commonly regarded as insoluble in water, their small solubility in water (benzol, 1.8 g./1000 g. water at 30° C., paradichlorobenzene, 0.08 g./1000 g. water at 30° C., monochlorobenzene 0.525 g./1000 g. water at 30° C.<sup>4</sup>) is, nevertheless, a necessary condition to their fungicidal effectiveness. Naphthalene could be expected to be ineffective because of its very low solubility in water and its low volatility.

It seems clear that these fumigant fungicides act through the medium of their aqueous solutions on and within the plant tissues, concentrations that are effective against the pathogen being lower than those that are toxic to the host. The ratio of distribution of the fumigant, between an aqueous phase and a lipoidal or waxy phase, is such as to greatly favor solubility in the non-aqueous phase. Transfer from the aqueous layers external to the cells into the lipoidal or waxy constituents of the cells occurs, even though the concentrations of volatile fungicide in the aqueous layers be small. As a result the cell constituents become profoundly modified and their permeability is broken down. Such a mechanism of action, to be effective, would require only very small amounts of the fumigant. The occurrence of such mode of action is indicated, as has been detailed, by plasmolysis of sporangia

<sup>4</sup> From unpublished data obtained by H. E. Vermillion, Duke University, Durham, North Carolina.

in aqueous solutions of benzol, by direct observations with the microscope, and by increase in conductance in host-tissue extracts.

Solution of fumigant in water has direct application to its effectiveness in seed bed treatment. The volatile substance is transferred through the air from its source within the bed, and dissolves in water on the plants, on the seed bed covers, and in the soil, thus becoming widely distributed over the bed area. This moisture, furthermore, acts as a fumigant-storage medium, since water has a greater capacity, per unit volume, than air for holding the substances herein under consideration. A liter of air, for example, saturated with benzol at 10° C. contains 0.199 g. of benzol, whereas a liter of water in equilibrium with benzol-saturated air will contain 1.76 g. The fumigant reevaporates more or less uniformly over the entire seed-bed area from its solution in films of water on the surfaces within the bed, thereby maintaining a concentration of vapor over the bed long after the liquid or solid fumigant in the applicators has vaporized.

#### SUMMARY

In some seasons benzol need not be applied every night in order to secure control of downy mildew of tobacco or to give complete protection against this disease. The length to which the interval between successive applications can be extended with safety is probably governed by the length of the sporangial cycle and by modificatory effects of prevailing weather conditions.

Cotton balls dipped into benzol constitute effective means for vaporization of this compound in seed beds.

As the result of exposure of tobacco seedlings to vapors of benzol and paradichlorobenzene there is an increase in permeability of the plasma membranes.

Concentrations of 1/16 saturated benzol, 1/2 saturated paradichlorobenzene, 1/750 saturated phenol, and 1/75 saturated aniline closely approximate the minimal toxic limits that inhibit germination of sporangia of *Peronospora tabacina*.

The principles involved in the use of volatile fungicides are briefly discussed in relation to their possible mode of action and to seed bed practice.

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## OBSERVATIONS ON TWO AMBROSIA BEETLES AND THEIR ASSOCIATED FUNGI<sup>1</sup>

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### INTRODUCTION

The symbiotic association of certain Scolytid beetles with the so-called "ambrosia" fungi has been recognized for nearly 100 years. There is, however, very little exact information about the nature of the association or about the ambrosia fungi. The beetles have been adequately described and classified by Hubbard (5), Swaine (15), and others, but the fungi have been very much neglected. Hartig, in 1844, described briefly the ambrosia fungus associated with *Xyleborus (Bostrychus) dispar* (Fabr.) and named it *Monilia candida*. Hubbard, in 1896, made an extensive study of the ambrosia beetles but included only casual observations on the associated fungi. He concluded, however, that there was more than one ambrosia fungus and that only the most closely related species of beetles cultivated the same one. The different fungi were not adequately described or differentiated by Hubbard, and no one ever has undertaken a comprehensive study of them.

During the summers of 1934, 1935, and 1936, two species of ambrosia beetles were very abundant on dying aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) in Itasca Park, Minnesota. *Trypodendron betulae* Sw. infested white birch and the aspen was infested by *T. retusum* Lec. The writers studied the life histories of the beetles and their associated fungi in nature, and the growth characteristics of the fungi in pure culture on artificial media. The work is not so extensive as would be desired and

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is by no means complete; but, since the authors will not have the opportunity of continuing the study, the data obtained are being placed on record with the hope that others may be encouraged to make more extensive studies of this interesting group of fungi.

#### AMBROSIA BEETLES AND AMBROSIA FUNGI

Schmidberger (11), as early as 1836, observed and described the association of beetles with the so-called "ambrosia." He observed that the beetle larvae fed upon a glistening white substance, already present in the galleries. Schmidberger did not understand the fungus nature of the substance, interpreting it as a product of the exuding sap. He applied the term "ambrosia" to it. Three years later these observations were confirmed by Ratzeburg (10), who suggested that the ambrosia was the product of a mixture of plant sap and insect spittle. The true nature of the ambrosia was first recognized in 1844 by Hartig (4), who described the fungus cultivated by *Xyleborus dispar* and named it *Monilia candida*. Although recognizing the ambrosia as a fungus, Hartig, believing in heterogenesis, concluded that it originated from the wood cells reacted upon by a substance secreted by the beetles.

For more than 60 years after Hartig's observations, the ambrosia fungi received little attention. From 1908 to 1913 Neger (6-9) and Schneider-Orelli (12) published a series of papers dealing with the subject. These investigators, working chiefly with species of *Xyloterus* and *Xyleborus*, described accurately the fungi associated with the beetles, grew some of them in artificial culture and attempted to explain the nature of the association.

According to Schneider-Orelli (12), the ambrosia fungus of *Xyleborus dispar* is transmitted to successive generations in the form of spores in the crop of the female beetle, which regurgitates them to start a culture in the new brood tunnel. Neger (9), however, thinks the spores are passed through the insect's body and survive in viable condition in pellets of excrement. Schneider-Orelli states that spores taken directly from the tunnels of *Xyleborus dispar* do not germinate, but if they are recovered from the crop of the female beetle they germinate readily.

Strohmeyer (14) has described several species of ambrosia beetles from specimens collected in the tropics. The female beetles of some of these species have special chitinous hooks or brushes on the front part of the head in which spores and mycelium of ambrosia are always found. These structures were interpreted as organs specialized for transporting the ambrosia fungus to new brood chambers.

Neger (7) expressed the opinion that the ambrosia fungus associated with *Xyleborus dispar* was an Endomycete, but neither he nor Schneider-Orelli reached a final conclusion as to the identity of the fungus. Trotter (16) recently reported the study of an ambrosia fungus associated with a species of *Xyleborus* infesting the wood of *Brownea grandiceps* Jacq. in Ceylon. He observed the "ambrosia" in material imported into Italy from

Ceylon and grew the fungus in hanging-drop cultures and observed its manner of fructification. In addition to a monilia-like growth, he observed a second layer of mycelium superimposed upon it. This second layer of mycelium formed an abundance of fusiform hyaline spores. On the basis of limited observations he concluded that the superimposed fungus was a second spore form of the first. For this supposedly pleomorphic ambrosia fungus he established a new genus, *Ambrosiamyces*, and named the type species *Ambrosiamyces zeylanicus*. The fungus differs widely from any other described ambrosia fungus.

The true ambrosia beetles belong to the family Scolytidae. They live in the sapwood, and occasionally the heartwood, of trees, infesting many different species. They are restricted mostly to weakened trees; vigorously growing trees or dead trees are rarely infested, although there are some exceptions. They bore deep into the sapwood, or even into the heartwood, each species making its own characteristic brood tunnels. The tunnels and breeding habits are of two general types. One group of genera is semi-social in habit, the beetles rearing their young in communal galleries, while in the other group each larva develops in its own separate larval chamber, excavating as it grows. The chief food of the developing larvae of both groups is the ambrosia fungus. According to Hubbard (5), the fungi associated with the beetles of the two groups may be separated into two corresponding groups on the basis of type of growth and method of spore formation.

In those genera in which the young are reared in a common gallery, the larvae have mouthparts that are not adapted to chewing wood and apparently they feed solely on the fungus. The larvae that develop in individual chambers have strong mandibles and consume wood, the fungus constituting only a part of their food. Although there is little or no experimental evidence, it usually is assumed that the ambrosia fungi, by concentrating the nitrogenous elements found in wood, provide a diet more suitable than wood alone.

The species of ambrosia beetles observed by the authors belong to the group in which the larvae are reared in individual chambers. No detailed descriptions of their life cycles or galleries have been published. The associated ambrosia fungi also have not been described in any detail, and there is no previous record of their culture on artificial media.

*Trypodendron retusum* Lec. This ambrosia beetle was very prevalent in dying aspen (*Populus tremuloides*) in Minnesota in 1934, 1935, and 1936, but was much less abundant in 1937 and 1938. It was not observed infesting any other species of tree. The beetles were never found infesting vigorous, rapidly growing trees or trees that had died the previous season. In nearly all cases infestation was confined to the lower half of the tree, being heaviest near the base and gradually diminishing towards the top. The upper limits of infestation usually coincided closely with the lower limit of living bark cambium. Attempts to start tunnels in living bark cambium usually are unsuccessful, the tunnels filling with sap and drowning the

beetles. Several healthy trees and infested trees were examined to determine the relation of moisture content of the wood to infestation. The water content of healthy trees averaged 65.7 per cent. That of the infested parts ranged from 70 to 80 per cent, while the upper noninfested part ranged from 60 to 70 per cent. The moisture determinations were made in June at the height of infestation. Later in the summer the trees died and the water content decreased to 50 per cent or lower, too low to permit fresh infestations. Trees with a second infestation seldom have been observed. Occasionally the beetles construct a second row of galleries beyond the first, but they have been observed only in trees with a D.B.H. of seven inches or more.



FIG. 1. Sections of brood galleries of *Trypodendron retusum* showing the larval cradles with the ambrosia fungus fruiting on the walls. A. A piece of aspen wood split through a gallery showing the vertical arrangement of the larval cradles opening off the main gallery. Two larvae are shown in place. The white ambrosia fungus may be seen fruiting on the walls of the lower cradles. Approx.  $\times 1$ . B. A closer view of the larval cradle showing the white masses of spores and sporophores of the ambrosia fungus. The main gallery is stained dark by the dark brown vegetative mycelium of the fungus. Approx.  $\times 4$ . C. Two pupae of *Trypodendron retusum* with heads toward the main gallery. Note the white masses of spores of the ambrosia fungus on the frass separating the larval cradle from the main gallery.

The overwintered beetles appear and become active in early spring and start making tunnels in the trees about the middle of May. In 1936, eggs were found in the galleries on May 23. Larvae in various stages of development were found throughout the month of June, pupation and transformation to adults occurring in early July. In late summer or early fall the new brood of adults leave the trees. They left the trees somewhat earlier in 1936 than in 1935. It was not determined where nor how the beetles survive the winter, but all available evidence indicates that they do not overwinter in the trees.

The galleries made by *Trypodendron retusum* are compound, dividing dichotomously once or twice in a horizontal plane. The single entrance tunnel extends from one-half to one-fourth inch into the wood before branching. The lateral tunnels follow a curved line parallel with the bark and usually less than one-half inch from the surface. The tunnel is excavated by the female, the male removing the accumulation of sawdust and frass. Usually 30 or more eggs are deposited in small niches at regular intervals along the roof and floor of the galleries, and each egg is covered with a small mass of sawdust and frass. The newly hatched larvae gnaw out cradles extending vertically at right angles to the main gallery, enlarging the cradles as they grow (Fig. 1, A). The sawdust and frass are forced out into the main gallery and are disposed of by the parent beetles.

*Trypodendron betulae* Sw. Paper birch (*Betula papyrifera*) was heavily infested with this species from 1934 to 1936, but few were found in 1937 and 1938. *T. betulae* is very similar to *T. retusum* in general appearance and life cycle, although there are a few minor differences in the construction of breeding tunnels. The tunnels of *T. betulae* extend somewhat deeper into the tree than those of *T. retusum*, usually penetrating well into the heartwood. The ambrosia fungi associated with the two beetles also were very similar, only minor differences between them having been observed. Dodge (3) reported that he had observed *T. retusum* tunneling in birch. The galleries were not completed, no eggs or larvae were found, but the fungus seemed to be able to grow satisfactorily.

The ambrosia fungi obviously are introduced into the tunnel by the adult beetles and often they may be found fruiting on the walls before the first egg has been deposited. The pads of frass covering the eggs always are permeated with the mycelium of the ambrosia fungus. On culturing the frass, the presence of contaminating fungi and bacteria can be demonstrated, but the mycelium of the ambrosia fungus always predominates. The walls of the larval cradles become overgrown with the fungus shortly after the eggs hatch (Fig. 1, A and B). The fungus layer is consumed quickly by the beetle as it enlarges its cradle, but a new crop of the fungus promptly appears. The fungus at first is glistening white, or cream colored, but the vegetative mycelium soon becomes darker. The hyaline, ovoid spores are borne in chains on short, simple sporophores arranged in a palisade layer. When a cross section of a larval chamber is examined under the microscope,

the spores and sporophores resemble the conidial stage of the powdery mildews (Fig. 2).

The mycelium penetrates the wood cells adjacent to the tunnel but rarely invades the wood for more than a few millimeters. The invading mycelium is dark-brown or black and stains the wood heavily, making the walls of the tunnels appear charred. The mycelium often fills almost the entire lumen of the cells but the walls apparently remain intact. The fungus probably obtains its nourishment from the cell contents and does not decay the cell walls. Sporulation is rare in the main galleries, and, if spores are pro-



FIG. 2. Photomicrograph of cross section through a larval cradle showing the sporophores of the fungus arranged in a palisade layer on the walls of the cradle. Approx.  $\times 150$ .

duced, they are promptly consumed or otherwise disposed of by the adult beetles.

The fact that contaminating fungi are present has been mentioned. As long as the tunnels are inhabited by the beetles, the ambrosia fungus predominates and is not overgrown by other fungi, but if the beetles die or leave the tunnels, the contaminating fungi promptly overgrow the ambrosia fungus. It is evident that the contaminating fungi are suppressed by the beetles, but the method of suppression was not determined.

The larvae may turn around in the cradles, but are usually found facing away from the main tunnel. Immediately before pupation the larvae reverse their position and face the main gallery. The pupae occupy this position during metamorphosis, and, on maturity, the new beetle emerges by eating out the plug of frass that separates it from the main gallery and on which the fungus usually is fruiting (Fig. 1, C). The new brood of beetles evidently acquires the fungus in this way, because it was determined by

examining stained sections of the insects that none of the consumed spores or mycelium survive metamorphosis within the pupae. Pupae in various stages of development have been cultured and examined histologically, but the fungus has never been found within the body. It was not definitely determined how the fungus survives within the beetles over winter, but it is believed by the authors that spores of the fungus remain in the intestinal tract during hibernation and are voided with excrement as the beetle begins to bore the new brood tunnel in the spring.

The fungi associated with the two species are very similar in appearance



FIG. 3. A. Monilioid sporulation of the ambrosia fungus associated with *Trypodendron betulae* when first isolated in pure culture on agar. Many of the spores or spore-like cells remained attached in chains. Only a small percentage were cut off. Approx.  $\times 320$ . B. Germinating spores of the ambrosia fungus produced in artificial culture. Germination of spores obtained directly from the tunnels was very erratic.

and have no characters that would justify considering them as distinct species. There was considerable variation in size of the individual spores in the tunnels but the differences were not consistent enough to be significant. On germination, the spores send out a simple germ tube, but germination was very erratic, those of the fungus associated with *Trypodendron betulae* germinating more freely than those associated with *T. retusum* (Fig. 3, B). Only minor differences were observed between cultures of the fungi on artificial media. The mycelium was at first hyaline but became brown with age and the medium was discolored by a diffusible brown stain. A dark brown liquid often was exuded from the mycelium and collected in drops on the surface of the colony. Most of the studies in artificial culture were made with the fungus obtained from the tunnel of *T. betulae* because of the relative ease with which pure cultures could be obtained.

When first grown in pure culture, the fungi sporulated very poorly, forming only imperfect monilioid spores that tended to remain attached and to bud *in situ*. All gradations between distinct spores and short roundish vegetative cells were observed (Fig. 3, A). After repeated subculturing, variants that sporulated freely arose as white patches or sectors on colonies grown on agar in Petri dishes. When the patches or sectors were subcultured, cultures that sporulated abundantly and consistently were obtained, although there was considerable variation in the general appearance of the colonies formed by different sectors (Fig. 4). The spores formed by the



FIG. 4. Five different growth types of the ambrosia fungus associated with *Trypodendron betulae* derived from sectors obtained after repeated subculturing. The white growth consists chiefly of sporophores that produce normal subspherical hyaline spores in abundance.

variants were hyaline, with thin walls, and appeared white in mass. They were ovoid to round, tending to be more nearly spherical than the spores produced within the insect tunnels in nature. They ranged from 6 to 17  $\mu$  in length and from 6 to 14  $\mu$  in width with average dimensions of 11.38  $\mu$  by 10.09  $\mu$ . The growth of the sporophores was indeterminate and the spores were cut off basipetally.

The rate of growth of the fungus on Petri dishes of potato-dextrose agar was determined at the following temperatures: 5, 10, 15, 20, 25, 27.5, 30, and 36 degrees C. The minimum temperature for growth was about 10° C., the optimum from 25 to 27.5° C., and the maximum between 30 and 36° C. The fungus grew very well at temperatures between 15 and 30° C.

No sexual reproduction of the fungus was observed in nature or in pure culture, and, obviously, it must be classified among the imperfect fungi. Some difficulty and uncertainty are faced in referring the fungus to a genus and species. It is clearly not the same fungus described by Trotter (16) as *Ambrosiamyces zelanicus* and, in so far as the writers have been able to learn, the only other name applied to an ambrosia fungus is that applied by Hartig to the fungus associated with *Xyleborus dispar*, namely *Monilia candida*. Although Hartig's description of *Monilia candida* is not very extensive, the same fungus has been well described by Schneider-Orelli. There is enough similarity between the fungus associated with *X. dispar* and the one under consideration to justify placing them in the same genus, but they are probably specifically different. The problem is complicated still further by the confusion that has surrounded the genus *Monilia*, and especially the binomial *Monilia candida*.

*Monilia* was first used as a generic name by Gmelin in 1791, but was redefined by Persoon in 1801. Persoon's description of the genus is given by Stevens (13) as follows: "Hyphae erect, branched, forming a dense mycelial felt, which produces numerous conidiophores, conidia catenulate, hyaline or light-colored, ovate or lemon shaped." Castellani (2) has referred to the genus *Monilia* a number of human pathogens having quite different characters. One of these was termed *Monilia candida*, apparently without knowledge of Hartig's prior use of the binomial. Berkout (1) made a study of the genera *Monilia*, *Oidium*, *Oospora*, and *Torula* and redefined them. The *Monilia candida* of Castellani was raised to generic rank and given the name *Candida* but unfortunately the *Monilia candida* of Hartig was overlooked or disregarded.

The ambrosia fungi associated with *Trypodendron betulae* and *Trypodendron retusum* apparently fall within the genus *Monilia* as defined by Persoon but they do not seem to be identical with *Monilia candida* Hartig. However, it is considered unwise to apply a new name to them until a relatively large number of the ambrosia fungi have been studied more thoroughly. Pending further studies they may be considered as strains of *Monilia candida* Hartig.

## SUMMARY

Two ambrosia beetles (*Trypodendron retusum* Sw. and *T. betulae* Lec.), affecting aspen and birch, respectively, and their associated ambrosia fungi, were studied and described. The two fungi that were grown in pure culture are considered to be very closely related strains of one species. This species probably is not identical with any previously named fungus but, because of the lack of any extensive study of the ambrosia fungi associated with other ambrosia beetles, it was not designated as a new species.

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### OVULINIA, A NEW GENERIC SEGREGATE FROM SCLEROTINIA

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In two preliminary reports (5, 6) and in an article now in press,<sup>1</sup> a destructive disease is described that affects the flowers of cultivated azaleas in the southeastern and southern United States, and the life history of the pathogen, a hitherto undescribed fungus of the *Sclerotinia* type, is outlined. The purpose of the present paper is to present a technical description of the fungus.

<sup>1</sup> A flower spot disease of cultivated azaleas. U. S. Dept. Agriculture Circ. 556, 1940.

The life cycle includes the following structures or stages: (1) apothecia, the ascospores of which initiate the primary flower infections; (2) conidia, which are produced on blighted flowers and infect other flowers, giving rise to successive flower infections and conidial generations throughout the period of azalea bloom; (3) microconidia (spermatia), which are produced on infected flowers in the late stages of their collapse, and coincidentally with (4) sclerotia, which are formed in the petal tissue following its complete invasion. The sclerotia may form in infected flowers subsequent to their abscission, or in flowers that collapse and persist on the branches. Ultimately, they reach the ground, where, lightly covered with soil or remaining on the surface, they give rise to apothecia at the beginning of the host blooming period in the following, or some subsequent, year.

The flower lesions resulting from infection by ascospores are similar to those described (*loc. cit.*, p. 236) as resulting from conidial infection, but their development in nature is typically less rapid, attributable probably to the lower optimum thermal range for ascospore infection (10 to 14° C.) as compared with conidial infection (15 to 20° C.). The ascospores germinate typically with 1, sometimes 2, polar germ tubes. Penetration of the cuticle of the azalea petal by the germ tubes is preceded by blanching of the petal color in a minute halo about the point of entrance and causes conspicuous tearing of the cuticle, indicative of forceful entrance (Fig. 3, D). The mycelium grows rapidly between the cells, causing rapid loss of coherence. The hyphae branch extensively, and the main strands attain a diameter of 8 to 12  $\mu$ . They are distantly septate (100 to 250  $\mu$ ) and densely protoplasmic in the juvenile portions. Ultimately the host cells become prevailingly free and suffer loss of color and structure, while the fungus permeates the fluid matrix derived from their disorganization. The petal tissue may retain a semblance of structure, chiefly through the support of the mycelium by the vascular elements and the cuticle, but when one pinches an infected petal lightly between the fingers, the tissue collapses and exudes sap. The expansion of a single lesion may be limited to 1 petal, but multiple infections typically result in invasion and collapse of the entire corolla.

Conidial production then begins, predominantly in the thin and delicate limbs of the corolla, and only to a limited extent in the corolla tube. Numerous short branches arise on the hyphae that complete the invasion of the host tissues; these become bifurcate and ultimately Y- or T-shape (Fig. 2, A, 1 and 4). The free ends enlarge, becoming globose or ovoid, and a septum forms across the branch just below the enlargement (Fig. 2, A, 2). A second septum then divides the apical portion into a small basal cell and a large conidium (Fig. 2, A, 3). The latter enlarges to its mature dimensions, while the basal cell ceases to grow and ultimately serves only as a disjunctor (Fig. 2, A, 3 and 4). By growth of the conidium and by extension of the branch that bears it, the conidia penetrate the cuticle, which becomes torn and shredded (Fig. 2, B). As the spore-bearing branches are produced in close proximity over the entire surface of the lesion, a mat or palisade of

conidia is formed on the surface of the petal (Fig. 1, B). They have been observed to number as many as 225 spores per sq. mm. of petal surface. Conidial production predominates on the upper (inner) surface of the petal, but may occur on the outer surface also, or anywhere on the corolla. Only 1 conidium is borne by each branch. Once the conidia are free above the surface of the petal, they are very readily detached from the conidiophores, bearing the disjunctor cell with them, so that they normally appear very unequally bicellular. As the disjunctor cell is empty, and often ruptures at or following abscission, the conidia are properly regarded as unicellular with a basal appendage.

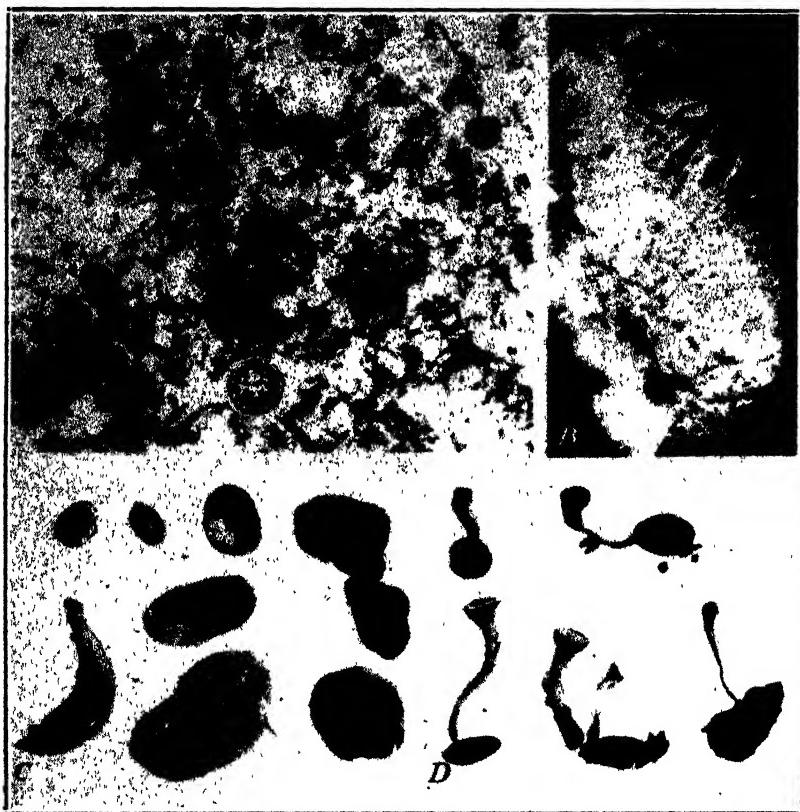


FIG. 1. A. Apothecia of *Ovulinia azaleae* emerging from sclerotia buried in sand.  $\times 2$ . B. Portion of azalea petal showing layer of conidia on or just above its surface, originating from subcuticular mycelium.  $\times 5$ . C. Sclerotia, showing cupulate form, smooth inner surface and verrucose outer surface.  $\times 5$ . D. Apothecia, showing occasional multiple stipes.  $\times 2$ .

The conidia are ellipsoid to ovoid or obovoid, with the base and apex equally convergent, or either may be larger and the other smaller (Fig. 3, C, 1). Sometimes (apparently associated with abnormally moist conditions) the conidia become greatly elongated, with a broadly clavate or pyriform shape (Fig. 3, C, 2). The conidia germinate promptly upon contact

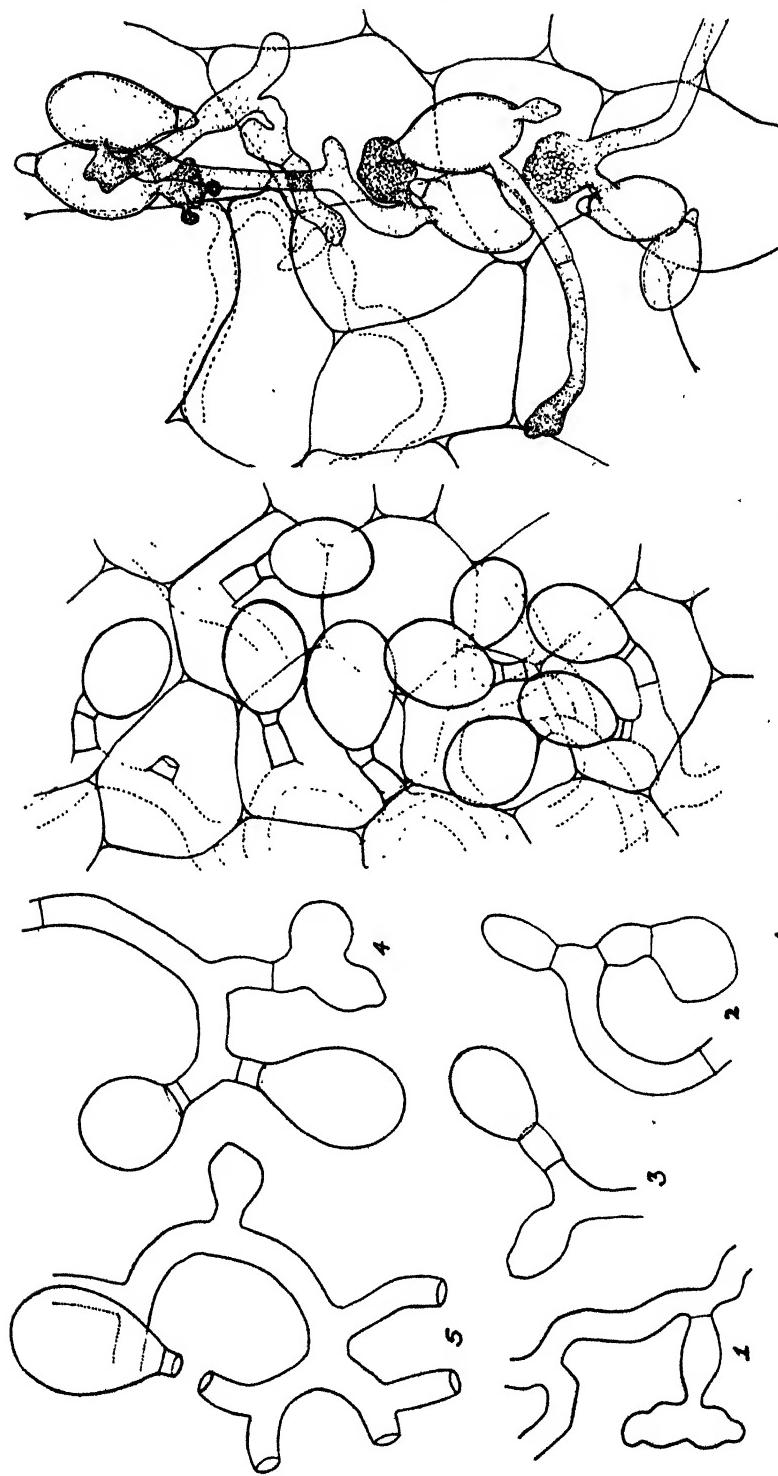


FIG. 2. A. Stages in development of conidia of *Ovulinia azaleae* on subcuticular mycelium in azalea petal. B. Group of mature conidia lying on surface of petal, showing conidiophores and vegetative hyphae. C. Germination of conidia and penetration of superficial cells of azalea petal by the germ tubes.  $\times 275$ .

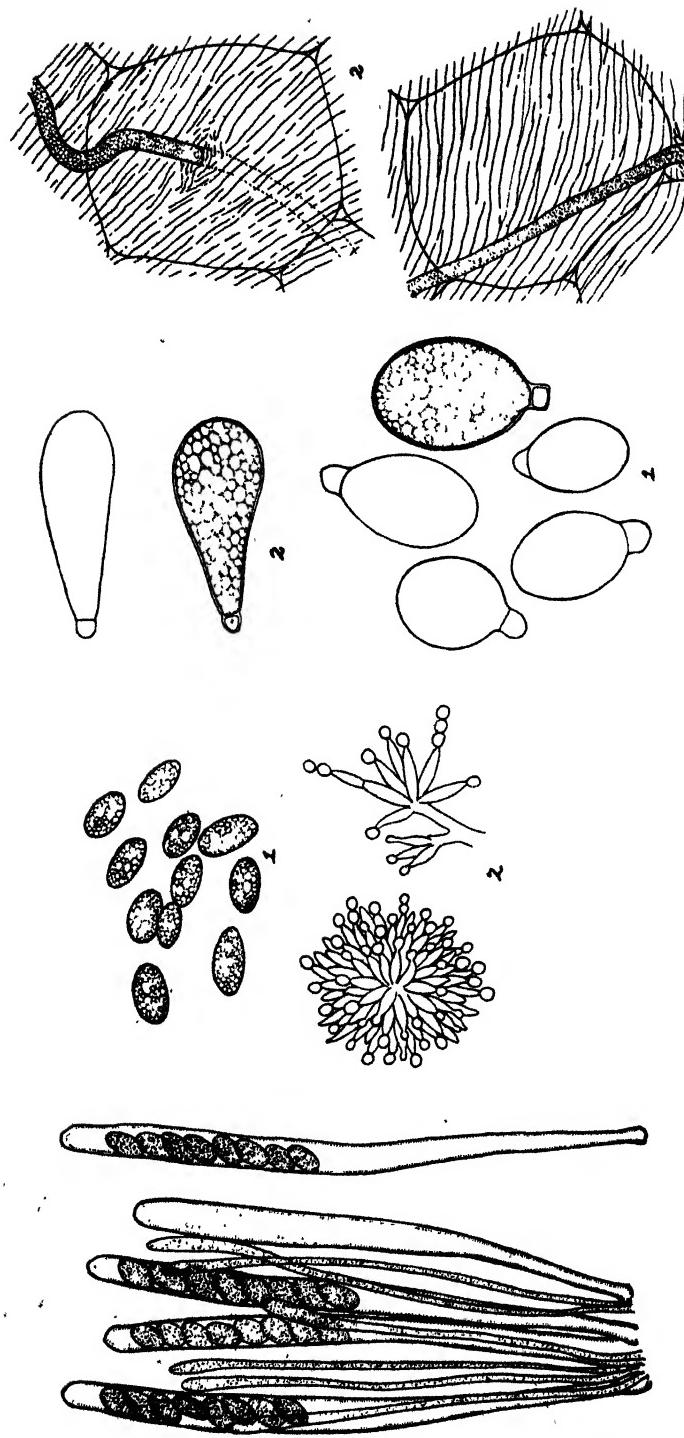


Fig. 3. A. Group of ascospores and paraphyses.  $\times 400$ . B1. Ascospores; B2. spernadochium and portion of same showing origin of spermata.  $\times 475$ . C. Normal conidia and elongated forms produced under excessive moisture.  $\times 400$ . D. Penetration of striate cuticle of azalea petal by ascospore germ tube, showing buckling of germ tube, rupture of cuticle, and pore left after passage.  $\times 400$ .

with water, usually with 1 germ tube opposite the basal cell, but often with multiple germ tubes from lateral positions. They often germinate in place in such numbers as to produce a macroscopically visible weft (Fig. 2, C). When kept dry, they may remain viable for 4 to 6 weeks at temperatures prevailing in nature during the period of the disease, and 8 to 12 months (rarely over 1 year) in artificial storage at 5 to 10° C. Their viability is not destroyed by severe freezing (-18° C.) under these conditions.

The so-called microconidia, actually spermatia (7) are produced in chains from cushions of short, fusoid hyphae (spermatiophores), the whole constituting a spermadochium, on the surface of the blighted flower (Fig. 3, B, 2). They are formed contemporaneously with the sclerotia.

The sclerotia first appear as blister-like swellings within the collapsed flower tissue; they are typically more numerous and larger in the thick tubular portion of the corolla. They are at first translucent and gradually darken and become black; they vary in shape from disciform to irregular, sometimes consisting of 2 or more portions joined by a narrow isthmus, and are distinctly cupped (Fig. 1, C). The convex side corresponds to the outer surface of the corolla. The concave side is smooth, and the convex side verrucose to rugose. From 1 to 20, commonly 2 to 5, sclerotia develop in a single flower. There is a distinct cortex and a medulla. The sclerotia are formed within the host tissue but are separate therefrom and appear to contain only fungus elements.

During the final stages of development of the sclerotia, they exude drops of a clear amber fluid. Minute spines are present on the margin of the sclerotium and distributed over the convex surface. One might suppose that the spines are receptive organs, the spermatia the male elements in a sexual cycle, and that the drops of fluid serve some purpose in promoting the fusion of the two, but no definite evidence of this nature has been obtained. Neither is it known whether the fungus is heterothallic, as definitely monosporous ascospore infections have not been obtained. However, monoclonial infections may give rise to both sclerotia and spermatia.

The sclerotia may produce apothecia after storage on slightly moistened peat and sand for 9 to 10 months or may remain dormant for 1 to 2 years and then give rise to normal apothecia. The temperature and other requirements for apothecial production have not been precisely ascertained, but there has been no consistent difference resulting from storage of the sclerotia at different temperatures between 5 and 18° C., though the actual production of apothecia has not occurred until the temperature was raised to 10-14 degrees. Freezing the mature, dry sclerotia (-18° C.) for several months did not destroy their vitality.

The fungus grows slowly on artificial media. On agar + sugar media it showed a preference for dextrose and a reaction approximating pH 6; but, on these substrates, it produces only a tough mat of grayish-white to buff mycelium, occasionally with sclerotium-like knots, which have not been induced to develop further. On vegetable media, such as steamed wheat or

barley, and on bean plugs, it produces sclerotia of more typical form and spermatia in abundance. These sclerotia have even borne spines similar to those formed in nature, but they have not yet been induced to produce apothecia. Conidial production has not been obtained in artificial cultures, but only on flowers following inoculation with ascospores, conidia, or mycelium.

#### DISCUSSION

The characteristics of the sclerotium and the apothecium obviously relate the fungus to *Sclerotinia*. In pathogenesis also it shows a marked similarity to the Vaccinium-infecting *Sclerotinia* spp. described by Woronin (8), Woronin and Nawaschin (9), and Fischer (1). None of these species is reported on azaleas, however, and they are described as developing only in the ovary (berry or capsule, depending on the host species) of infected flowers. Their conidial stages are all of the *Monilia* type.

In manner of conidium production the azalea fungus differs from any form as yet connected with *Sclerotinia* (4). Honey (2, 3) listed the macroconidial stages of *Sclerotinia* as belonging to the following types:

1. Macroconidia in chains
  - a. Disjunctors present
  - b. Disjunctors absent
2. Botrytis-like
3. Penicillium-like

His genus *Monilinia* (2) was erected to include the species of *Sclerotinia* having macroconidia in chains. The only additional genus since segregated from the Ciborioideae (subfamily including *Ciboria* and *Sclerotinia*) is Whetzel's *Septotinia* (7), which differs widely from any of the above in its septate conidia and branched conidiophores.

There being no generic type in this grouping that produces conidia singly on simple conidiophores, the writer, following the lead of Honey and of Whetzel, proposes the erection of a new genus, for which Whetzel has suggested the name *Ovulinia*, with the type species *O. azaleae*.

It may be noted here that a fungus referable to *Botrytis cinerea* very commonly occurs on azalea flowers that have been damaged by abrasion, frost, or beating rain. It forms sclerotia not only in the petal tissue, similarly to *Ovulinia*, but also in the calyx, capsule, filaments, and style, where the sclerotia of *Ovulinia* have not been found. The globose or thick sclerotia of *Botrytis* are readily distinguishable from those of *Ovulinia*, which are always flat or cupulate. The writer also has collected an apothecium of the *Ciboria* type, arising from a pseudosclerotium formed within the anthers of cultivated azaleas, which had overwintered on the ground. This apothecium differs distinctly in macroscopic and microscopic characters from the apothecium of *Ovulinia azaleae* and a separate description will be given.

#### OVULINIA, N. GEN.

Apothecia *Sclerotiniæ* similis, e sclerotii orientia; asci tenues, cylindrici, inoperculati; ascopora 8, ellipsoideæ, unicellulares, hyalines, uniseriatae; paraphyses ple-

rumque simplices, teretes, apicibus inflatae; conidia magna, obovoidea, unicellularia, appendicula basali praedita, hyalina, solitaria ex apicibus ramulorum brevium simplicium nata; spermatia (microconidia) minuta, globosa, in catenulas in hyphis brevibus fusoideis caespites formantibus nata; sclerotia disciformia vel irregularia, tenuia, leniter cupulata, nigra.

Apothecia of the Sclerotinia type, arising singly or in groups from sclerotia. Asci slender, cylindrical, inoperculate; ascospores ellipsoid, 1-celled, hyaline, typically 8 in 1 series; paraphyses mostly simple, terete, tips swollen.

Conidia large, obovoid, 1-celled, with a basal appendage consisting of a sterile disjunctor cell, hyaline, produced singly at the tips of short, simple branches from a parasitic mycelium within, and forming a mat on the surface of the host organ.

Spermatia (microconidia) minute, globose, produced in chains on short fusoid hyphae forming tufts (spermadochia) on the surface of the host, accompanying the formation of sclerotia.

Sclerotia disciform to irregular, thin, shallowly cupulate, black, formed within but discrete from the host tissues.

#### OVULINIA AZALEAE<sup>2</sup>

Apotheciorum solitariis vel 2-3-caespitosis ex quoque sclerotio, stipitatibus, urecolatis vel cyathiformibus, dein applanatis, 2-5 mm. latis, fulvo-olivaceis usque brunneis, margine seabooso, granuloso vel hirsuto; stipite 2-3 mm. (interdum 15-18 mm.) longo, 1-1.5 mm. crasso, recto vel leniter curvato, ad basim argillaceo, apicem versus cinnamomeo, fere glabro; hymenio brunneo, pruinoso; ascis cylindricis, 140-260  $\mu$  longis, 9-14  $\mu$  crassis; ascoporis 8, uniseriatibus, ellipsoideis, unicellularibus, 10-18  $\times$  8.5-10  $\mu$ , hyalinis, 1.3-guttulatis; paraphysibus teretibus, septatis, plerumque non ramosis, apice leniter inflatis; conidiis obovoideis, hyalinis, appendicula basali inclusa 40-60  $\times$  21-36  $\mu$ , solitariis ex apicibus ramorum brevium simplicium natis, de conidioforis e cellula disjunctori conidio affixa manentei separantibus; spermatia globosis, 3.0-3.5  $\mu$  diam., apicibus hypharum fusoidearum 10-12  $\mu$  longarum, 3  $\mu$  crassarum, in caespites aggregatarum orientibus; sclerotis disciformibus vel irregularibus, cupulatis, nigris, 2-5  $\times$  3-10  $\times$  0.5-1.5 mm. Parasitica in floribus Rhododendri spp. e Carolina superiore usque Texas, U. S. A., etiam flores Rhododendri, Kalmiae, et Vaccinii spp. artificiosae inficiens.

Apothecia arising singly or in groups of 2 to 3 (rarely up to 8) from the margin of a sclerotium (Fig. 1, A, D), lying on or shallowly covered with soil, in late winter and early spring, stipitate, ureolate to cyathiform, flat at maturity, 2-5 mm. broad, tawny olive to snuff brown, margin scaly, granuloso or hirsute; stipe typically 2-3 mm. long, 1-1.5 mm. thick, erect or slightly curved, but sometimes sinuous, filiform, and up to 15-18 mm. long, clay-color (R) at the base, darkening to cinnamon at the top, glabrous, rarely with 1 or a few rhizoids; hymenial surface russet to walnut brown, somewhat pruinose. Asci cylindrical, 140-260  $\mu$  (average 180  $\mu$ ) long by 9-14  $\mu$  (average 12  $\mu$ ) thick; apical plug not staining blue with iodine (Fig. 3, A). Ascospores 8, uniseriate, ellipsoid, 1-celled, 10-18  $\times$  8.5-10  $\mu$  (average 16.3  $\times$  9.3  $\mu$ ), hyaline, usually with 1-3 prominent globules (Fig. 3, B, 1). Paraphyses terete, septate, mostly unbranched, apices slightly swollen.

Conidia typically obovoid, hyaline, 40-60  $\times$  21-36  $\mu$  (average 50  $\times$  28) including the basal appendage; when formed under high humidity becoming clavate to pyriform, up to 72  $\mu$  long; produced singly on short simple branches protruding from the host surface and arising from a parasitic mycelium underneath; separating from the conidio-phores by means of a disjunctor cell which remains attached to the conidium (Fig. 2, A; 3, C). This stage forms a thin mat or weft on the surface of the host organ and the conidia are promptly disseminated therefrom by insects and meteoric water or germinate in place.

Spermatia globose, 3.0-3.5  $\mu$  in diameter, produced at the tips of fusoid hyphae, 10-12  $\times$  3  $\mu$ , which are aggregated into minute tufts (just visible by a 10  $\times$  lens) on the host surface (Fig. 3, B, 2); usually separating readily but sometimes adhering in short chains; appearing coincidently with sclerotia.

Sclerotia formed within invaded host tissues but separable therefrom when mature; typically of circular to elliptical outline, often irregular, distinctly cupped, smooth on the concave surface, verrucose to rugose on the convex (Fig. 1, C); 2-5  $\times$  3-10  $\times$  0.5-1.5 mm.; cortex and medulla differentiated structurally but at maturity black throughout.

Growing readily on 2 per cent potato-dextrose agar at pH 6.0 when recently isolated, forming a coarse, tough, mat-like mycelium, grayish white to pale fawn in color, becoming stromatoid and dark in color with age and tending to be short-lived. On vegetable media (bean pods, barley or wheat kernels) sclerotia and spermatia are also formed, but no macroconidia. Optimum temperature for growth 18 to 22° C.

<sup>2</sup> I am indebted to Miss Edith K. Cash for the preparation of the Latin diagnosis.

The ascospores and conidia infect flowers of cultivated azaleas and rhododendrons (*Rhododendron mucronatum*, *R. pulchrum*, *R. simsii*, *R. obtusum*, and *R. catawbiense*) causing a destructive flower blight in the southern and southeastern United States from North Carolina to Texas; pathogenic experimentally to a wider host range, including the flowers of native azaleas of the eastern United States, *Kalmia* and *Vaccinium*.

**Herbarium material:** The type specimens are deposited in the Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture, at Washington, D. C., under Nos. 71105-71108. Duplicate material is deposited in the Plant Pathology Herbarium at Cornell University, Ithaca, New York.

#### DIVISION OF MYCOLOGY AND DISEASE SURVEY.

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#### INVASION OF SWEET-CORN PLANTS OF DIFFERENT AGES BY STRAINS OF PHYTOMONAS STEWARTI

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(Accepted for publication October 16, 1939)

The sweet-corn wilt bacterium, *Phytomonas stewarti* (E. F. S.) Bergey *et al.*, is usually more destructive on young seedlings than on older plants. It produces large leaf lesions, causes diffuse wilting, and usually kills the seedlings, as described by Ivanoff (2). Older plants may be extensively invaded but are less severely injured. Seedlings are very susceptible, but some of those that are not killed outright recover, continue growth, and become sufficiently resistant with age to produce mature plants, as observed by Wellhausen (9). This observation suggests that either the morphology or physiology of maize changes sufficiently to alter the host-parasite relationship as the plant grows beyond the seedling stage, or the bacteria are modified in old plants.

It was considered of interest to test the virulence of several strains of *Phytomonas stewarti* for plants of different ages. The strains selected for these studies were B-11, the single-colony isolates obtained from it and numbered B-1011, B-1211, and B-1111, and several single-colony isolates obtained from strain B-1111. The origin, virulence for young seedlings, and physiological characteristics of B-11, B-1011, B-1211, and B-1111 have been

TABLE 1.—*Invasiveness of 10 strains of Phytomonas stewarti in sweet-corn plants inoculated at different ages*

Strain tested	Age of test plants	First test			Second test			Average infection index in 2 tests		
		Condition of leaves		Infection index	Condition of leaves		Infection index			
		Total no.	No. dead		Total no.	No. dead				
<i>Days</i>										
B-1011	7	59	5	.41	.95	55	8	.54	1.42	1.18
	14	70	1	.66	.99	71	5	.60	1.06	1.02
	19	83	0	.72	.87	86	0	.88	1.02	.95
	24	110	0	118	1.07	103	0	.83	.81	.94
B-11	7	59	6	.41	1.00	61	2	.46	.85	.93
	14	68	1	.58	.90	67	2	.61	1.00	.95
	19	84	0	.72	.86	85	0	.70	.82	.84
	24	101	0	93	.92	99	0	.95	.96	.94
B-1211	7	62	0	0	.00	64	0	1	.01	.01
	14	76	0	4	.05	75	0	7	.09	.07
	19	88	0	12	.14	86	0	16	.19	.16
	24	105	0	29	.28	98	0	19	.20	.24
B-1111	7	66	0	2	.03	60	0	4	.07	.05
	14	75	0	7	.09	72	0	9	.12	.11
	19	83	0	31	.37	85	0	30	.35	.36
	24	103	0	28	.27	103	0	87	.85	.56
B-1111-8	7	65	0	0	.00	61	0	2	.03	.02
	14	72	0	5	.07	77	0	4	.05	.06
	19	85	0	10	.12	89	0	15	.17	.14
	24	96	0	47	.49	103	0	50	.49	.49
B-1111-11	7	66	0	1	.01	63	0	5	.08	.05
	14	75	0	6	.08	72	0	7	.10	.09
	19	84	0	18	.21	85	0	16	.19	.20
	24	103	0	35	.34	98	0	35	.36	.35
B-1111-3	7	65	0	7	.11	63	0	1	.01	.06
	14	76	0	14	.18	75	0	2	.03	.11
	19	86	0	17	.20	82	0	13	.16	.18
	24	101	0	45	.45	97	0	25	.26	.35
B-1111-5	7	63	0	5	.08	58	0	6	.10	.09
	14	73	0	14	.19	73	0	6	.08	.14
	19	86	0	16	.19	87	0	17	.20	.19
	24	100	0	18	.18	99	0	13	.13	.15
B-1111-6	7	62	0	13	.21	65	0	9	.14	.17
	14	71	0	18	.25	72	0	9	.12	.19
	19	83	0	10	.12	85	0	21	.25	.19
	24	107	0	58	.54	100	0	36	.36	.45
B-1111-14	7	60	0	39	.65	52	1	27	.58	.62
	14	74	0	35	.47	71	0	29	.41	.44
	19	85	0	39	.46	87	0	53	.61	.53
	24	103	0	28	.27	95	0	34	.36	.31

described elsewhere (5, 6). The single-colony isolates obtained from B-1111 were similar to the parent culture except for differences in virulence. At the time the present tests for virulence were made, strains B-11 and B-1011 were capable of using inorganic nitrogen very readily; B-1111-14 used it sparingly, and the remaining cultures used it with difficulty, if at all. In other words, the weakly virulent cultures employed in these tests differed from the virulent ones in that they required organic nitrogen for their growth.

A nutrient-dextrose broth subculture of each strain was inoculated into 7-, 14-, 19-, and 24-day-old sweet-corn plants of the variety Golden Bantam. Duplicate groups of 10 plants each in the 4 age groups were inoculated with each strain. Each plant was injected at the crown and at 2 points along the leaf whorl. The plants were observed for infection 13 days after inoculation and records were taken for the calculation of an infection index based upon the average number of necrotic lesions produced per leaf (5). The data obtained and presented in table 1 show that, although there were some minor variations, the duplicate tests agreed very closely.

The highly virulent strain B-1011 was very invasive on plants of all age groups. Although the averaged data for the 2 tests show that the culture produced fewer lesions on the older plants, the differences are not statistically significant. The virulent strain B-11 and moderately virulent B-1111-14 gave similar results. On the other hand, the weakly virulent

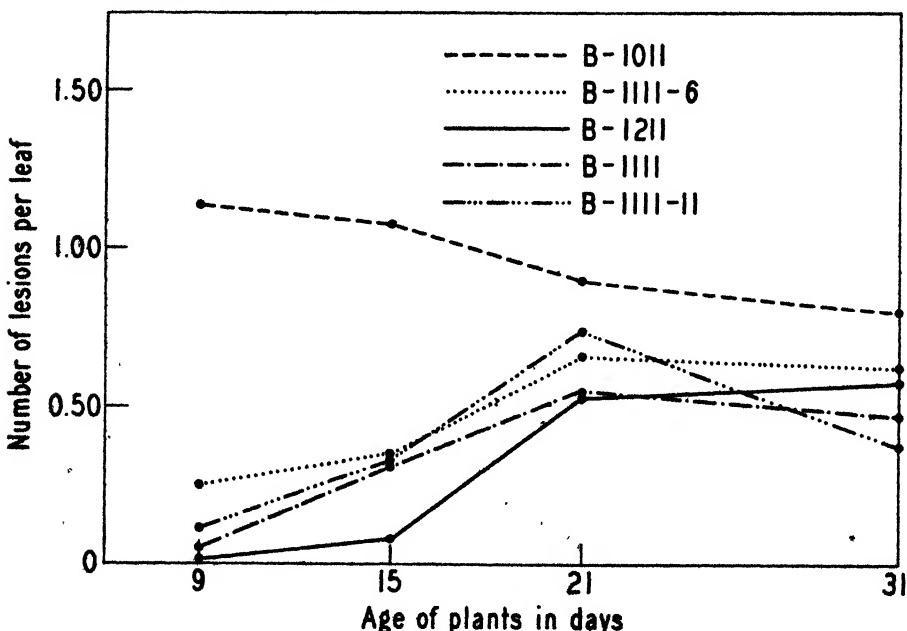


FIG. 1. Average number of necrotic lesions produced by different strains of *Phytoponas stewarti* on leaves of sweet-corn plants of different ages. There was a tendency for strain B-1011 to produce fewer lesions on the leaves of older plants even though it did cause a general wilting. The strains that were almost avirulent for young seedlings caused distinct lesions in plants over 15 days old.

strains were, without exception, more invasive in the 2 older groups of plants. They produced necrotic and chlorotic streaks on leaves of the 19- and 24-day-old plants, even though they usually failed to cause visible lesions on 7-day plants. The lesions produced ordinarily were not so large as those caused by the more virulent strains, and, consequently, the plants were not so severely injured.

A repetition of this test using B-1111-14, B-1111-11, B-1111-6, B-1111-3, B-1111, B-1211, B-1011, and B-11 on 9-, 15-, 21-, 31-, and 57-day-old plants gave comparable results. The data obtained on B-1011, B-1211, B-1111, B-1111-6, and B-1111-11 are presented graphically in figure 1. The weakly virulent strains produced more lesions on the older plants than on the younger ones. The data on the 57-day-old group are not presented in figure 1 because these plants, which were in tassel at the time of inoculation, did not show distinct lesions. However, the plants were invaded extensively by both the weakly virulent and highly virulent strains and showed considerable general wilting. The most severely invaded leaves from representative plants of the 9-, 21-, and 31-day-old groups are illustrated in figure 2.

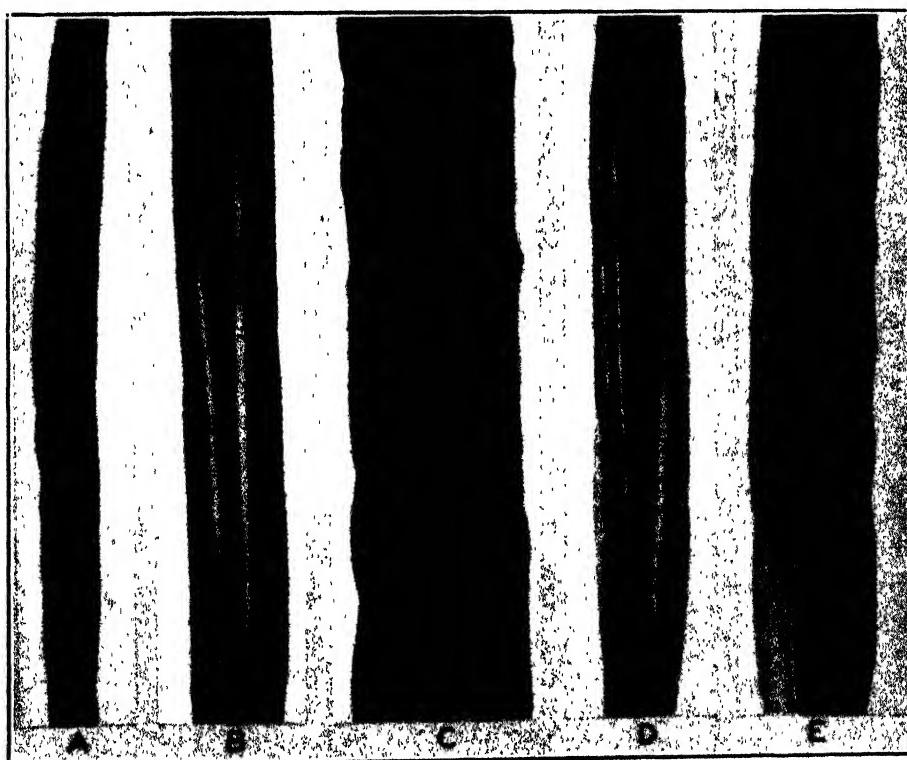


FIG. 2. Leaves from sweet-corn plants inoculated with strain B-1211 when the plants were 9 (A), 21 (B), and 31 (C) days old and with B-1011 when they were 21 (D) and 31 (E) days old. Each leaf was the most severely invaded one from a typical plant in each age group. (Photograph by J. A. Carlile.)

In order to determine whether the virulence of the strains was changed by incubation in the host, bacteria were recovered from leaf lesions and stems of several plants in each age group and tested for virulence on 10-day-old seedlings. The data obtained confirmed those reported previously (6) and may be summarized as follows. Cultures B-11 and B-1011 were not definitely affected but some of the isolates from plants inoculated with B-1111-14 were more virulent than the strain injected. Isolates obtained from the necrotic lesions on 19- and 24-day-old plants inoculated with strains B-1111 and B-1211 were not appreciably more virulent than the parent cultures. However, virulent isolates were observed in 1 of the 6 groups tested. Some of the bacteria recovered from the stems of 24-day-old plants severely invaded by B-1111 were almost as virulent as B-11. These virulent derivatives from B-1111 were found to be capable of utilizing inorganic nitrogen when transferred to the synthetic medium used for such tests (6).

#### DISCUSSION

The data presented in this paper show that the relationship of host and parasite changes as sweet-corn plants grow older. The highly virulent strains produce no more lesions on old plants than on young ones, but the weakly virulent strains become distinctly more invasive as the plant grows beyond the seedling stage. The obvious conclusion is that there is some change in the host's physiology that affects the weak strains' ability to multiply. The nature of this change is not immediately obvious, but, as mentioned before, it may be assumed that the change affected either the host's susceptibility or the bacteria's virulence. The latter possibility can be dismissed because weakly virulent strains were recovered from the lesions they had produced. The fact that only weakly virulent strains were isolated from some plants argues against the idea that the bacteria had been changed before they invaded. The virulent isolates obtained from some plants inoculated with weakly virulent strains apparently developed at random in the host, just as they do in cultures (6) of the weakly virulent strains.

There is no direct evidence of a change in the growing plant that would permit B-1211 and B-1111 to become invasive as the plant ages. Since it has been shown (6) that the impotency of these strains is caused by their inability to grow on inorganic nitrogen, a logical assumption to explain the observations reported above would be that organic nitrogen appears in the tracheal tubes as the plant passes from the seedling to the independent stage. There is not enough known about translocation of organic nitrogen in maize to verify this assumption. It has been shown that practically all of the reserve proteins in the seed are converted into soluble compounds (3) and are transported to the growing tips of the plumule and root (7, 8), where they accumulate as asparagine or combined proteins (7) within the first week after the seed begins to germinate. Protein nitrogen from the seed, therefore, does not enter into the present discussion because the period of infection was 2 to 4 weeks after germination. Any organic nitrogen that

reaches the xylem during this later period must be synthesized by the plant from carbohydrates and inorganic nitrogen. Such a synthesis might very well be delayed until after the second week of growth when the plant had produced sufficient leaf area to manufacture carbohydrates. If so, the change in host metabolism must have occurred at the same time the host became more susceptible to strains such as B-1211. The organic nitrogen produced by the growing plant may have passed from the phloem into the adjacent xylem tissue. At least carbohydrates reach the xylem in sufficient quantity to support bacterial growth. It is also known that organic nitrogen occurs in the tracheal sap of other plants (1, 4).

#### SUMMARY

Virulent strains of *Phytomonas stewarti* were as invasive on young sweet-corn seedlings as on more mature plants. Weakly virulent strains, on the other hand, were much more invasive on plants that were over 14 days old than on younger plants. Since these weakly virulent strains were obligate users of organic nitrogen, the hypothesis is advanced that organic nitrogenous compounds appear in the tracheal tubes after the plant has become established and has started synthesizing its own organic materials.

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# PREVALENCE OF CUCUMBER AND TULIP VIRUSES IN LILIES

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Until recently, lily mosaic was believed to be caused by a single virus, peculiar to lily and of very limited host range. In 1937, McWhorter (3) demonstrated that a virus of the tulip group occurs commonly in symptomless lilies of certain species, and Price (5) isolated a strain of *Cucumis Virus I* from Easter lily (*Lilium longiflorum* Thunb.) showing typical necrotic fleck symptoms. The writer (1, 2) has presented evidence that the viruses described by McWhorter and by Price are distinct, and that viruses of both these classes can be recovered from the typical necrotic fleck type (Fig. 1, B) in Easter lily. However, the strong mottle in Easter lily (Fig. 1, A) has yielded only a tulip virus. In commercial forcing of Easter lilies only fleck symptoms are commonly interpreted as mosaic, since plants carrying strong mottle, or carrying only McWhorter's latent virus, are satisfactory for forcing.

McWhorter demonstrated the existence of his latent virus of lily by inoculating the juice of lilies into healthy tulips by hypodermic needle. We have compared this tulip test with that of rubbing young leaves of *Lilium formosanum* Stapf. Parallel trials of 23 individual Easter lily plants by these two methods are compared in table 1. Symptoms in *L. formosanum*

TABLE 1.—Comparative results of inoculating *Clara Butt* tulips and *Lilium formosanum* seedlings as index plants for tulip virus in Easter lilies

Source plant			Results of inoculating	
Number	Description	Symptoms	Tulips	<i>L. formosanum</i>
C <sub>1</sub> 37-1	Creole	None	4/6	4/4
C <sub>2</sub> 37-1	do	do	3/6	3/4
F 23-1	do	do	0/6	4/5
C <sub>1</sub> 37-39	do	Mottle	4/6	10/10
C <sub>1</sub> 37-17	do	Fleck	1/6	3/4
C <sub>1</sub> 37-113	do	do	4/6	4/4
Ct 36-33	Croft	None	5/6	1/4
Ct 37-16	do	do	1/6	2/4
Ct 37-24	do	do	0/6	3/4
Ct 37-74	do	do	0/6	0/4
E <sub>1</sub> 37-241	Erabu	do	2/6	4/4
E <sub>2</sub> 37-26	do	do	4/6	3/4
E <sub>3</sub> 37-48	do	do	0/6	2/4
G 37-48	Giganteum	do	1/6	3/4
G 37-63	do	do	2/6	4/4
H 37-136	Harrisi	do	3/6	4/4
H 37-123	do	Mild mottle	4/6	4/4
WQ 7	White Queen	None (seedling)	0/6	0/4
WQ 142	do	do	0/6	0/4
152-2C-1	Seedling	None	0/6	0/4
152-2T-9	do	do	0/6	0/4
152-3C-7	do	do	0/6	0/4
GS 85-7	do	do	0/6	0/4

<sup>1</sup> Number of plants infected over number inoculated.

were recognizable in 10 to 14 days after inoculation; those in tulips were expressed in the year following inoculation. It appears from these data that *L. formosanum* is a satisfactory index plant for tulip virus in lilies. The agreement between tulip and *L. formosanum* as test plants is reasonably close with a few discrepancies suggesting that the lily test is more efficient

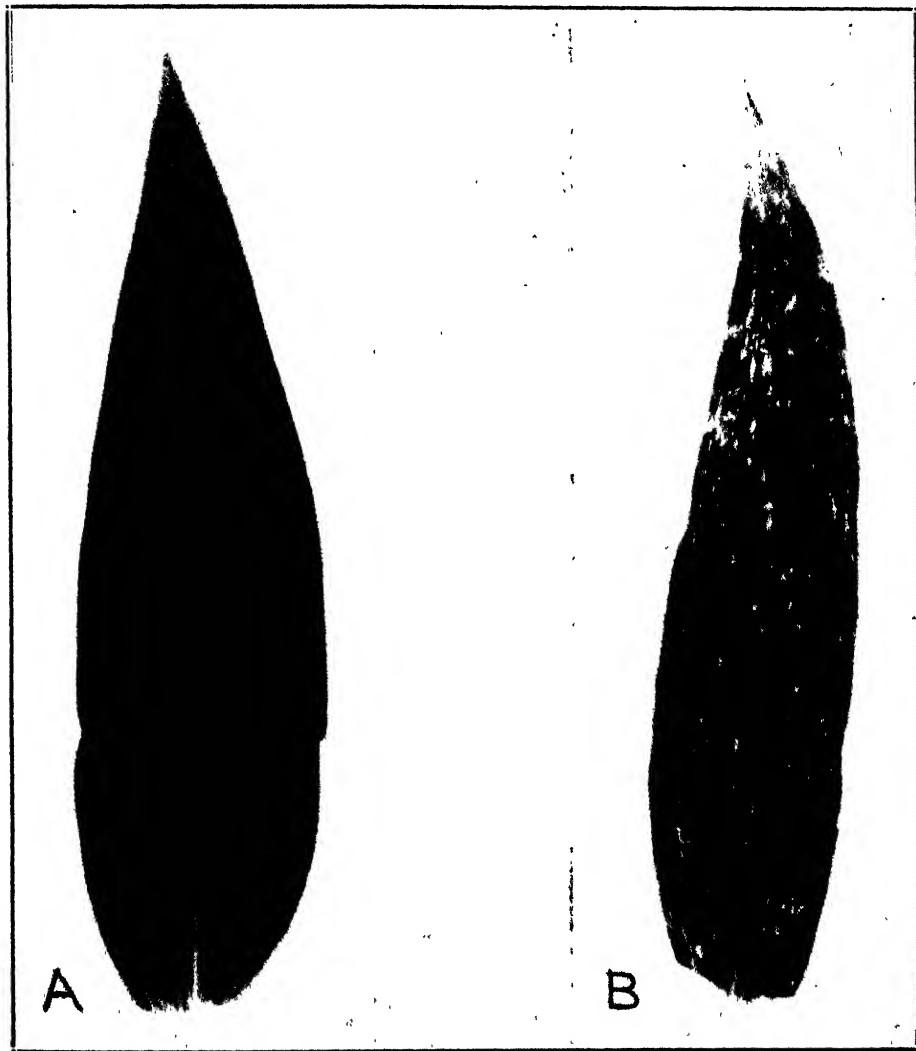


FIG. 1. Mosaic symptoms in *Lilium longiflorum* var. Creole. A. Strong mottle. B. Typical necrotic fleck.

than the tulip test, as well as much faster. It is not yet known whether these discrepancies represent merely differences in the efficiency of the methods in our hands or a differential susceptibility of the test plants.

The Easter lilies tested in the trials listed in table 1 include symptomless selections from Louisiana Creole, Florida Creole, Oregon Croft, Japa-

nese Erabu and Giganteum, and Bermuda Harrisi. The individual source plants were selected during the forcing of 200 to 600 of each commercial variety, as plants free from virus symptoms throughout the forcing period. The fact that all but one of these symptomless lilies showed evidence of latent tulip virus strongly suggests that this virus is present in nearly all commercial Easter lilies from all sources. At the same time 6 green-house-grown seedlings tested virus-free on both tulip and *Lilium formosanum*.

Price (5) used Turkish tobacco as a test plant in demonstrating cucumber virus in lilies. We have adopted this test and, combining it with the *Lilium formosanum* test, have used a simple mechanical inoculation of these 2 species as an index of the presence of cucumber and tulip viruses in lilies. A representative set of plants for such indexing is shown in figure 2, A, and another set is shown in figure 2, B, 9 days after inoculation from a specimen of the hybrid lily George C. Creelman, which proved a carrier of both tulip and cucumber viruses. Carborundum dust was applied to the leaves rubbed in all inoculations. Turkish tobacco is inoculated while very small, as shown in figure 2, A. *L. formosanum* responds satisfactorily at any stage of active growth when new leaves are developing. We have regularly washed off the inoculum shortly after the operation is finished, since the juice of lilies will often burn tobacco leaves if allowed to dry in place.

Infection in Turkish tobacco is considered evidence that a cucumber virus is present. Symptoms usually appear as white necrotic rings in 4 to 6 days, and may or may not be followed by systemic mottling. If no infection appears in tobacco, but *Lilium formosanum* develops yellowing and curling of the young leaves in 6 to 10 days, followed by mottling of various types, a tulip virus is considered present. If both test species respond, as shown in figure 2, B, the plant indexed carries both viruses. During hot summer weather *L. formosanum* may develop a mild mottling in young leaves from cucumber mosaic alone, but this can be distinguished from tulip virus on subsequent development.

It has not yet been proved that all viruses isolated from lily and that produce symptoms in Turkish tobacco belong to Cucumis Virus I, but it seems probable that they will be so classed. There is less reason for regarding as strains of a single virus all collections that are positive in *Lilium formosanum* and negative in tobacco, but this working hypothesis is maintained for the present. The possibility remains that other viruses not detected by this index test occur in lily, but the conception of 2 distinct viruses, each including a number of strains, seems adequate to account for most of the virus patterns thus far encountered in our study. The "yellow flat or rosette type" (4) has not been recognized in our material.

During the summer of 1939, garden lilies of many species and varieties from a number of localities were indexed by the method described above. Material was collected by the writer and by S. L. Emsweller, or mailed to this station by E. P. Imle and others. Five plants of each test species were used in each index trial. In 56 representative trials in which tulip virus

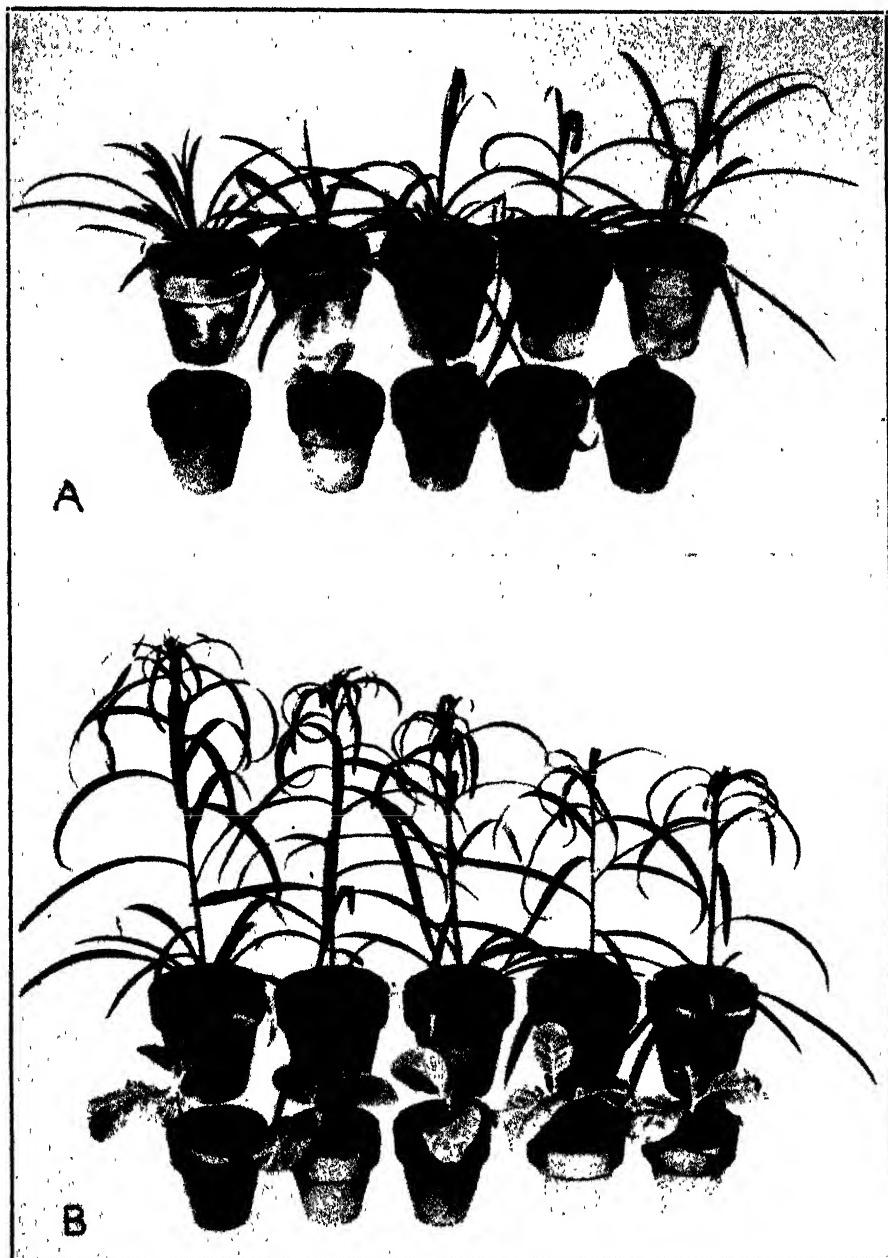


FIG. 2. Test plants used in detecting tulip and cucumber viruses in lily. *Lilium formosanum* seedlings in 4-inch pots and Turkish tobacco in 3-inch pots. A. Representative set at stage suitable for test. B. A set 9 days after inoculation from a plant of the hybrid lily George C. Creelman, showing symptoms of tulip virus in *L. formosanum* and of cucumber virus in tobacco.

was detected the mean number of *Lilium formosanum* plants showing symptoms was  $4.14 \pm .15$ . In 27 trials in which cucumber mosaic was found, the

TABLE 2.—Results of indexing garden lilies for the presence of cucumber virus (C) and tulip virus (T) by inoculation into *Turkish tobacco* and *Lilium formosanum* seedlings. Figures indicate number of plants indexed. C + T indicates both types of virus found. H indicates plants apparently virus free.

Species or variety	Source locality					
	Yonkers, N. Y.	White Plains, N. Y.	Chinotette, Vt.	Ottawa, Canada	Ithaca, N. Y.	Geneva, N. Y.
Amabile luteum	T	T	2T	2C+T 1C	H	
Auratum and var. <sup>a</sup>	T	10H	H	C+T 1C	T	
Backhouse hybrids				C		
Brownii				2T		
Canadense (incl. var.)				10H	T	
Candidum	C+T	C+T	T	C+T	T	T
Cernuum		C		H		
Chalcedonicum						
Groenii	T					
David (incl. Willmottiae)						
Davnoftiae						
Dauricum luteum						
Elegans (and seedlings)						
Formosanum	T	12H	T	2T	3T	
George C. Creelman		5H	10H	T	T	
Giganteum						
Hansonii						
Hanson; Marhan hybrids	H					
Henry						
Leucanthum						
Maxwell	T	C				
Monadelphum		C				
						2T

TABLE 2.—(Continued)

Species or variety	Source locality		T		Corallines, Ore.	
<i>Myriophyllum superbum</i> ( <i>Sulphureum</i> )	Yonkers, N. Y.	T	C	T	C + T	C
<i>Nepalense</i>	White Plains, N. Y.	T	C + T	C + T	2T	2H
<i>Pardalinum giganteum</i>	Charlottesville, Va.	T	C + T	C + T	1T	1H
<i>Principis</i> (and hybrids)	Ottawa, Canada	T	C + T	C + T	10 + H	H
<i>Pinnatum</i> ( <i>tenuifolium</i> )	Ithaca, N. Y.		2C + T	T	1C + T	C
<i>Regale</i> (and hybrids)	Glengary, N. Y.		C + T	T	1H	H
<i>Sagittariae</i> (and hybrids)	Battsville, Md.		H	H		
Speciosum and vars.	Thurmont, Md.		H	H		
<i>Superbum</i>	Aspen, Md.		H	H		
<i>T. A. Havemeyer</i>	Bellmgham, Wash.		T	T		
<i>Testaceum</i>	Belleview, Wash.					
<i>Trigynnum</i>						
<i>Umbellatum</i>						
Wallacei (and hybrids)						
Unnamed hybrids						

Also tulip virus, San Francisco.

Also apparently virus-free at Ogallala, Nebr., and Mission Bottom, Oreg.

mean number of Turkish tobacco plants infected was  $4.70 \pm .14$ . Since the general level of infection is high in those sets that proved positive, some confidence may be placed in the significance of negative readings.

The results of these garden-lily trials are summarized in table 2. Tulip virus was detected in 31 species or varieties of lilies in 41 indexed, and from 13 localities in 15 sampled. Cucumber virus was found present in 18 species or varieties from 9 localities. Cucumber virus was not detected in 22 collections representing 6 localities in the West. Possibly the later season of indexing (July 28 to August 8), or the longer time in transit, was unfavorable for detecting cucumber virus in western samples, but the result is a striking one. Cucumber virus was found occurring alone in 11 index trials representing 10 species and varieties and 6 localities.

Twenty-two collections, including plants of 14 species or varieties from 7 localities, were found carrying both tulip and cucumber viruses. Some of these double infections were found in crook-neck *Lilium auratum* Lindl. and similarly affected *L. superbum* L.; in other instances *L. regale* Wils., *L. sargentiae* Wils., *L. tigrinum* Ker, and *L. umbellatum* Hort. carried virus of both classes with less damaging effects, although usually somewhat dwarfed.

Several of the bulb-propagated species, commonly assumed to be carriers of virus, were sampled in these trials. *Lilium candidum* L. in 6 samples carried tulip virus, in 2 samples both tulip and cucumber viruses, and 10 plants of recent seed origin were found virus-free. The hybrid George C. Creelman carried tulip virus in 4 localities and both viruses in 1 locality. *L. elegans* Thunb., of supposed hybrid origin, and commonly represented by named varieties, carried tulip virus in all 5 samples tested. The hybrids Maxwill, Princeps, T. A. Havemeyer, and *L. testaceum* Lindl., were all affected with one or more viruses in 8 tests. *L. tigrinum* was found affected with tulip virus 9 times, with both types once, and tested virus-free 8 times in samples from 7 localities. *L. tigrinum* was found apparently healthy in isolated gardens and in material recently collected from such isolated sites. *L. umbellatum*, of supposed hybrid origin and, like *L. elegans*, commonly represented by named varieties, carried one or both viruses in 5 samples, and was apparently healthy in 2 trials.

*Lilium hansonii* Leichtl., its hybrids Marhan (*L. martagon album*  $\times$  *L. hansonii*), and the Backhouse hybrids (Martagon-Hansonii hybrids), were uniformly virus-free in 9 tests that represented 50 individual plants from 5 localities. This species, as well as its hybrids, is vegetatively propagated, and is known as a type not subject to mosaic. These tests show that it is not commonly a symptomless carrier but may be resistant to infection or may tend to escape.

No virus was detected in leaves from bulbs of *Lilium myriophyllum superbum* (Baker) Wils. and *L. nepalense* D. Don, grown in a greenhouse at Geneva, New York, and said to be collected from the wild in India. This finding is in agreement with a general view that bulbs from the wild are virus-free.

A few conclusions may be drawn from the data available here. The existence of apparently virus-free *Lilium tigrinum* is in itself some assurance that isolation may be effective in protecting lilies from virus infection. Moreover, where isolation of garden-lily seedlings has been conscientiously attempted, it appears successful, as far as our sampling and indexing can be considered conclusive. Isolation has proved effective thus far in protecting our plantings of *L. longiflorum* seedlings at Charleston, South Carolina, for 2 years and at Los Angeles, California, for 1 year. The consistent failure to detect virus in *L. hansonii* and its hybrids, even when long grown in close proximity to diseased lilies, indicates that this species carries either resistance to or some tendency to escape from mosaic. The detection of double infections in *L. candidum*, George C. Creelman, *L. regale*, and *L. sargentiae* hybrids at White Plains, N. Y., in a private-garden collection, long carefully maintained at a high level of performance, suggests that there is not a close correlation between double infections and poor performance in all species. It may be inferred further that hybrids of the Regale-Sargentiae class may be carriers of complexes, as well as single virus infections, without showing symptoms that would commonly call for roguing.

These index tests show that both tulip virus and cucumber virus are involved in mosaic of garden lilies. As in Easter lilies, the tulip virus is more commonly found. The occurrence of 2 distinct viruses in garden lilies greatly complicates problems involving resistance, tolerance, or capacity to escape infection. Obviously, the symptoms found in lilies in nature must be reproduced in inoculation experiments before the viruses isolated can be established as the sole causal agents. Determination of the reactions of the various species and varieties to each and to both viruses will be a long and tedious undertaking. In the meantime the culture of lilies from seed under suitable isolation offers promise as a solution of the problem of virus-disease control. Those who attempt to grow virus-free lilies by this method must face the fact that either tulip virus or cucumber virus may occur in latent form in appropriate species that have not been suitably isolated.

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# THE RELATION OF TEMPERATURE TO COMMON AND HALO BLIGHT OF BEANS<sup>1</sup>

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Inoculation experiments with common blight of beans (*Phaseolus vulgaris* L.) caused by *Phytononas phaseoli* (E.F.S.) Bergey *et al.* and halo blight caused by *Phyt. medicaginis* var. *phaseolicola* Burk. have resulted in considerable variation in the percentage of infection, time of appearance of symptoms and the type of symptom occurring. This variability has been evident both in the field and in the greenhouse, where conditions were more uniform. Differences in the age and variety of bean plants being tested failed to adequately explain the variability. It was also observed that these diseases sometimes occurred in commercial fields with apparent suddenness and under conditions that did not seem especially favorable.

The literature contains many statements based on observational evidence concerning the conditions most favorable for the two diseases. In general, common blight is considered as a high-temperature disease. Burkholder (1) observed the disease was more prevalent in hot than in cool seasons and that infection was more easily obtained in the greenhouse at 80° than at 65° F. He noted that at the latter temperature the leaf spots developed very slowly. From observational evidence he concluded that, "The pathogene progresses more rapidly in the plant tissue at high temperature than at low." Halo blight is generally considered a low-temperature disease. Burkholder (1) describes two types of leaf spots, the halo spots, which he found to occur during cool weather, and small, numerous angular lesions without a definite halo, which he observed to occur later in the season, during hot weather. He considered the lack of a halo to be due partly to the fact that the whole leaf became chlorotic.

Moisture usually is considered as being essential for both diseases, although Muncie (3) states that common blight may spread rapidly during dry weather. Higgins (2) considers moisture to be essential for infection with halo blight, and points out that "Infection is very materially reduced and often entirely inhibited if the plants are allowed to dry to the wilting point even three or four days after inoculation." Zaumeyer (4) tested the effect of high moisture, both before and after inoculation, by the use of moist chambers. He found that very little infection occurred with either disease unless the moist chambers were used for 24 hours after inoculation.

Because of the lack of published, experimental evidence the following experiments were undertaken to establish more accurately the effect of temperature upon infection and upon the subsequent development of these diseases. A few tests were included to determine the possible effect of rela-

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tive humidity upon the development of these diseases after infection had occurred.

#### METHODS

The experiments were carried out in electrically heated, double-wall glass cases in a refrigerated greenhouse. These cases were placed over water baths and an electric fan circulated the air over the water, so that a high relative humidity could be maintained. In some of the experiments a contrasting low relative humidity was obtained by eliminating the water bath.

Because of their known susceptibility to both bacterial blights, Red Kidney beans, obtained from a blight-free area, were used in all of the tests. These were grown in galvanized boxes or cans and the soil moisture was kept near the optimum as judged by the appearance of the plants. In each test the plants were selected for uniformity of size and development, the first trifoliolate leaf being well developed and the second and third just unfolding.

The plants were placed in the high-humidity cases for 24 hours before being inoculated. The upper and lower surfaces of the leaves were then thoroughly sprayed with a bacterial suspension made up of a mixture of strains of the organism to be tested.

Noninoculated controls were not included in the tests conducted in the temperature cases, as it was considered that the leaves produced after the plants had been inoculated would serve as an adequate check on the effect of temperature on the foliage. These leaves almost invariably remained free from blight. Additional plants from the same seed source were always grown in another greenhouse at the same time and always remained free from both halo and common blight. The bean plants grew slowly at 12° C. but the foliage appeared normal. The best growth occurred at temperatures of 16°, 20°, and 24° C. There was considerable etiolation at 28° and very poor growth with considerable yellowing at 32° C.

#### RESULTS

*Relative Humidity.* In the first experiments with each disease, after an incubation period of 24 hours at a high relative humidity, one-half of the plants at each temperature were removed to a low-relative-humidity case held at the same temperature.

Low humidity with common blight caused the infected area of the leaf to dry out more rapidly and the area affected was greater than at high humidities. This greater severity of the symptoms with low humidities occurred at all of the temperatures tested. In contrast with common blight, the low humidities had no apparent effect on the development of halo-blight symptoms. Even at the high temperatures, where the foliage sometimes reached the wilting point in the low humidity cases, the severity of infection was as great as with high humidity. This is in contrast to the results reported by Higgins (2).

*Temperature. Common Blight.* In the experiments referred to above it was found that temperature had a very marked effect on the length of the

incubation period with common blight. The first symptoms appeared in 6 days at 32°, 10 days at 28°, 14 days at 24°, and no symptoms were evident at the end of 17 days at 20° and 16° C. It was at first thought that no infection had occurred at these low temperatures, but, when, after 17 days, the plants at 20° were transferred to 32° C., large blighted areas appeared on the leaves in 2 days. Those at 16° were transferred to 28° C. and symptoms appeared 7 days later. The rapidity with which the symptoms developed when the plants at 20° were transferred to 32° C. clearly indicated that infection had occurred at the lower temperature, but that the symptoms were masked. In addition to the longer incubation period, the symptoms were always less severe at the low temperatures, particularly on the primary leaves. It was noticeable at all temperatures that infection was more prevalent on the older leaves than on those that were just unfolding at the time of inoculation.

The results of this first experiment suggested that the symptoms would have appeared if the plants had been held longer at 16° and 20° C. Accordingly, the experiment was repeated several times for longer periods, using only the high relative humidity chambers. The results presented in table 1 are of a typical experiment. Symptoms developed at 20° after 23 days

TABLE 1.—*Relation of temperature to common blight*

Tem- per- ture ° C.	Num- ber of plants	Days to first symp- toms	Infection of primary leaves				Infection of trifoliolate leaves <sup>c</sup>			
			None	Slight	Me- dium	Severe	None	Slight	Me- dium	Severe
32	14	7	35	38	23	4	65	17	12	6
28	23	9	46	31	13	10	59	21	13	7
24	24	16	73	23	4	0	47	29	13	11
20	12	23	96	4	0	0	55	34	8	3
20-32 <sup>a</sup>	11	23 <sup>a</sup>	68	32	0	0	40	20	10	30
16	12	27	100	0	0	0	94	6	0	0
16-32 <sup>b</sup>	9	27 <sup>b</sup>	92	6	0	0	74	21	5	0

<sup>a</sup> The temperature was changed from 20° to 32° C. 23 days after inoculation at which time there were no symptoms on the primary leaves and only 12 per cent of the secondary leaves showed slight infection.

<sup>b</sup> The temperature was changed from 16° to 32° C. 23 days after inoculation at which time there were no symptoms. The symptoms appeared 4 days after changing to 32° C.

<sup>c</sup> Records are based on final readings made 27 days after inoculation on the first 3 trifoliolate leaves. The degree of severity indicates the relative amount of infected tissue per leaf.

and a few symptoms appeared on plants held at 16° C. after 27 days. The symptoms of plants transferred from these temperatures to 32° rapidly increased in severity, although those originally held at 16° C. never developed symptoms so severe as those at higher temperatures during the duration of the experiment (27 days).

The causal organism was consistently isolated from leaves showing symp-

toms including those where the symptoms had previously been masked by low temperatures.

The above experiments were all conducted with the Red Kidney variety. It is possible that other varieties might vary in their response to temperature, but a few tests made with the Great Northern variety of field beans yielded results similar to those reported for the Red Kidney variety.

*Halo Blight.* In the preliminary experiments the first symptoms of halo blight appeared in 6 to 10 days after inoculation at 24° and 28° C. Usually the symptoms appeared 2 to 3 days later at the lower temperatures, but the effect of temperature on the incubation period was very slight as compared with common blight. As with common blight, there was considerable variation in the percentage of leaves infected at the different temperatures, but no consistent differences could be noted within a single experiment.

The effect of high temperature was chiefly confined to a modification of the symptoms and to an increase in the number of infection points per leaf. As previously stated, the relative humidity after a 24-hour period following inoculation had no effect on the development of the disease. The experiment was repeated several times, using a high humidity at all temperatures, and the effect of temperature was similar in all experiments. The results of 3 such tests are combined in the data presented in table 2. The amount of

TABLE 2.—*Relation of temperature to halo blight*

Temperature ° C.	Number of plants	Infection of trifoliolate leaves <sup>b</sup>				Occurrence of halo symptom <sup>c</sup>
		None	Slight	Medium	Severe	
° C.	Number	Per cent	Per cent	Per cent	Per cent	
32	46	55	10	11	24	none
28	48	60	18	13	9	none
24	63	66	19	11	4	small
20	63	64	28	5	3	large
16	62	58	32	7	3	large
12 <sup>a</sup>	21	77	18	3	2	large

\* Plants grown at 12° C. were from a different experiment but were grown in a comparable manner.

<sup>b</sup> The data are based on the trifoliolate leaves exposed at the time of inoculation and the results of three experiments are averaged. The degree of severity indicates the relative number of infections per leaf.

<sup>c</sup> See figure 1 for type of infection.

infection on the primary or cotyledonary leaves was very small as contrasted with that occurring with common blight. Because of this small amount of infection and the lack of consistent differences, the data on primary leaves are omitted from the table.

The two outstanding effects of temperature were the increased number of infections per leaf at high temperatures and the variation in the type of symptoms. In table 2 it can be seen that a higher percentage of leaves was listed as severely infected at the high temperatures. The degree of severity was based on the number of infections.

The typical halo surrounding the infected area occurred only at the lower

temperatures, 12°, 16°, and 20° C., at all of which temperatures there were typically few infection points per leaf, but the light green-yellow halo often involved one-fourth or more of the leaf area. At 24° the halo was quite small, and at 28° and 32° C. there was no halo visible to the naked eye. The symptoms at these two high temperatures on Red Kidney plants were so atypical that they could easily be mistaken for insect injury. The spots were usually less than a millimeter in diameter on the upper surface and

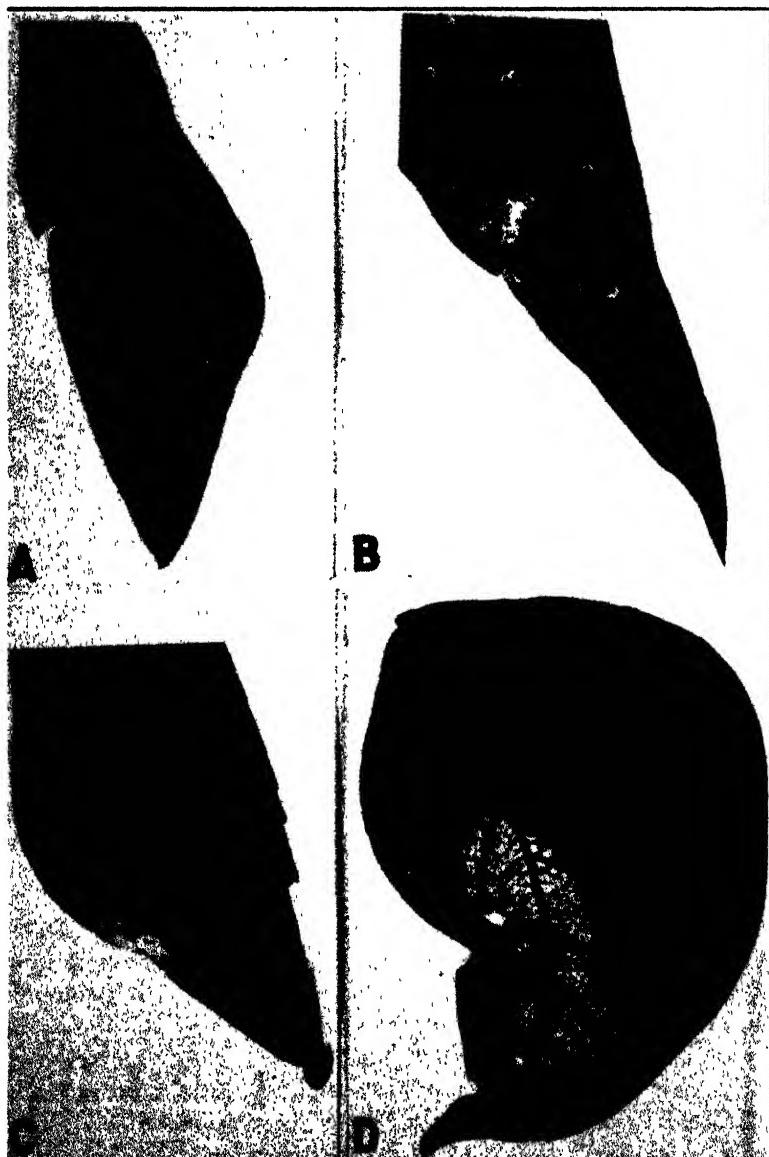


FIG. 1. The effect of temperature on the symptoms of halo blight of beans. A, B, and C. Portions of inoculated leaves at 28°, 24°, and 20° C., respectively. D. Inoculated leaf at 16° C.

slightly larger on the under side of the leaf. When magnified a very narrow band of chlorotic tissue could be seen (Fig. 1) on the upper side of the leaf, but it in no way resembled the halo that occurred at the lower temperatures. Bacterial exudate was usually present on the under side of the infected spots. Figure 1 shows typical examples of the variation in symptoms occurring at different temperatures. The causal organism was repeatedly isolated from the bacterial exudate of these atypical spots, which occurred at high temperatures.

The lack of a halo around the infected tissue was not due to a general chlorosis of the leaf, as noted by Burkholder (1). Chlorosis often is associated with a very large number of infections per leaf, but no halo occurred at high temperatures when only one or two infections were present on a green leaf.

In all of the halo blight inoculations it was noted that the greatest number of infections occurred on the youngest leaves that were just unfolding at the time of inoculation. This was in contrast with common blight, where the most infection occurred on the older leaves.

One test was conducted in which U. S. No. 5 Refugee was compared with the Red Kidney. The effect of temperature on the symptoms was similar, but it is possible that other varieties might differ in their response to temperature.

#### DISCUSSION

While common blight can be termed a high-temperature disease, due to the rapidity with which a large amount of leaf tissue can be involved, it is clearly evident that infection can occur at relatively low temperatures for the bean plant and that the incubation period is greatly prolonged at these low temperatures. This delay in the appearance of symptoms might possibly explain some outbreaks of the disease under conditions usually not considered ideal for infection. The rapidity with which the symptoms develop when transferred from low to high temperatures indicates that this is a masking effect and not entirely a slowing up of the progress of the pathogen through the plant tissues, as stated by Burkholder (1). Further experiments are necessary to determine how much of the delay in the appearance of symptoms is attributable to a slower invasion and how much to the masking of symptoms in invaded tissue.

Halo blight, likewise, can occur over a wide range of temperatures. The occurrence of the conspicuous halo at the lower temperatures readily explains why this disease is considered to be favored by low temperatures. The actual number of infections, however, may be greater at higher temperatures, and each of these inconspicuous spots can serve as a source of inoculum for the further spread of the disease. It would be difficult to detect many of these lesions in a casual examination of plants in the field. An epidemic could be gradually built up in this way, so that, with later weather conditions favorable for the development of the halo symptom, one might believe a severe infection had occurred simultaneously in the entire field.

## SUMMARY

Common blight of beans was produced by inoculation with *Phytomonas phaseoli* at all temperatures tested from 16° to 32° C. At low temperatures the incubation period was greatly prolonged. At 32° the symptoms appeared in 7 days, at 20° symptoms appeared in 23 days, while at 16° C. only slight symptoms appeared on a few leaves at the end of 27 days.

With common blight the transfer of plants from low to high temperatures caused the rapid development of symptoms on leaves previously appearing healthy. The amount of affected leaf tissue was greatest on the plants held constantly at the high temperatures. The oldest leaves of the young plants tested were more susceptible to infection than the young leaves just unfolding at the time of inoculation.

Halo blight was produced by inoculations with *Phytomonas medicaginis* v. *phascolicola* at all temperatures tested between 12° and 32° C.

The conspicuous halo occurred chiefly at 20° and below and, occasionally, a slight halo was present at 24° C. At 28° and 32° C. no halo symptoms appeared, the infections were greater in number, but the spots were small and inconspicuous, although bacterial exudate was present on the under side of the leaves.

The youngest leaves, just unfolding at the time of inoculation, were the most susceptible to halo blight.

Very little infection of primary leaves occurred with halo blight, while, with common blight, they were seriously infected.

The relative humidity after a 24-hour incubation period at high humidity had no effect on halo blight, but low humidities increased the severity of the symptoms on leaves infected with common blight.

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## SPORANGIAL PROLIFERATION IN PERONOSPORA TABACINA

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To date, no one has been able to grow members of the family Peronosporaceae, or downy mildews, on artificial media. In order to have available an adequate supply of sporangia for our studies of *Peronospora tabacina* Adam, frequent transfer of the pathogen from diseased to healthy seedlings proved necessary. These seedlings were grown in half-gallon glass fruit

jars containing approximately 500 g. of previously steam-sterilized soil. Seedlings were inoculated by atomizing a water suspension of sporangia upon them, and the jars were then closed with their screw-cap tops. The jars were then placed in a special incubation chamber maintained at a temperature of approximately 15° C. and continuously illuminated with a 25-watt bulb. Under these abnormal conditions, the pathogen produced proliferated sporangia. This phenomenon is recorded, for, so far as the writers are aware, it has not hitherto been recorded for any of the Peronosporaceae.

It should be borne in mind that *Peronospora tabacina* and other species of the genus possess dendritic, dichotomously-branched sporangiophores and that the sporangia are borne at the apices of curved, tapering branchlets. All sporangia of *P. tabacina* mature and are shed almost simultaneously, presumably being dislodged by air currents or by rain. Under usual conditions a crop of sporangia is produced at daybreak each morning, a response probably governed by light conditions. The sporangia range in size from  $10.5-24 \times 10.5-22 \mu$ , the average dimensions being  $18.4 \times 15 \mu$  (13).

Under the conditions of sustained high relative humidity within the jars and of constant weak illumination to which the pathogen and seedlings were exposed, the diurnal rhythm in production of sporangia was interrupted. Instead of finding fully developed sporangia in the morning, it was noted that the sporangiophores emerging from the several adjacent stomates were in different stages of development, the terminal branchlets often being more than twice as long as normal. The sporangia varied greatly in size, remained attached to the sporangiophores, and several types of proliferation were in evidence. In some the inner sporangial wall protruded apically and became enlarged (Fig. 1, *a* and *b*). In others there was formed a tube that either remained unbranched or branched dichotomously, developing small secondary terminal sporangia (Fig. 1, *c*, *d*, and *g*). In consequence a primary sporangium might give rise to 1 to 4 secondary sporangia, each smaller than the primary sporangium. As indicated in figure 1, *f*, the secondary sporangia also may proliferate, and tertiary sporangia would, no doubt, eventually be formed in such cases. Proliferation of another type is shown in figures 1, *h*, wherein the primary sporangium has produced a sporangiophore, dichotomously-branched and bearing eight diminutive, immature, secondary sporangia.

Proliferation apparently is causally related to the continuously high relative humidity and subdued illumination to which the pathogen was subjected. This opinion is supported by the fact that during the 8 years in which this fungus has been studied proliferation has never been noted on naturally infected plants grown out-of-doors.

In other fungi closely related to *Peronospora tabacina*, including members of the families Saprolegniaceae and Pythiaceae, the phenomenon of proliferation is not of unusual occurrence. In the former family, among species of *Saprolegnia*, new sporangia may form repeatedly within the basal

portion of the old sporangium; in the latter family a similar condition exists in the case of *Pythiomorpha gonapodiooides* Petersen (8). This type of proliferation involves the portion of the hypha immediately beneath the sporangium and not the sporangium itself. Kanouse (6) observed that in *P. gonapodiooides* the apex of the sporangiophore may be rejuvenated repeatedly, resulting in series of sporangia arranged nest-like or in a row. Butler's

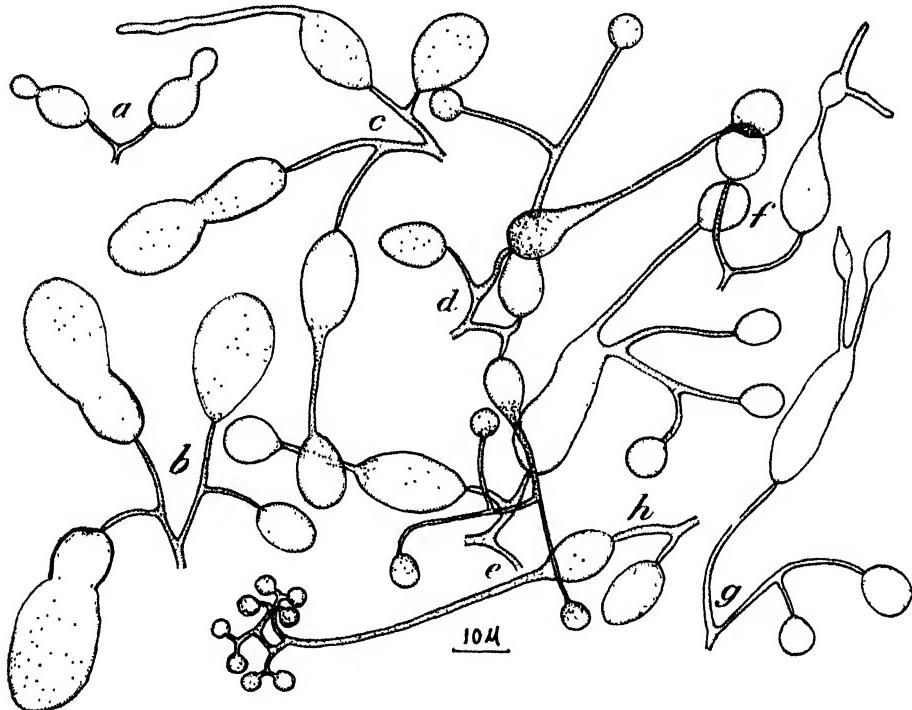


FIG. 1. *a*. Two dwarfed sporangia, each of which has budded in a yeast like manner. *b*. Four sporangia, one dwarfed, one of normal size, and two approximately twice the normal length, whose inner wall has become extended from the sporangial apex. The sporangiophore tips are abnormally elongated. *c*. Group of four sporangia, one of which has sprouted preparatory to forming a secondary sporangium, and another of which is forming a secondary sporangium. *d*. One sporangium has grown to form a secondary sporangium, another is forming 2 diminutive sporangia, and another one is forming three tiny sporangia. *e*. Proliferation of sporangia one of which has produced a single sporangium, the other of which, abnormal in size and shape, has proliferated to form four sporangia. *f*. The secondary sporangium is itself proliferating. *g*. The secondary sporangia borne by one elongated sporangium in process of formation. *h*. One sporangium that has proliferated to form a sporangiophore on which eight sporangia are forming.

plate 3, figure 4 (1) shows, in *Pythium proliferum* de Bary, a series of proliferating sporangia growing through and one above the other.

Another type of proliferation, involving sporangiospores instead of sporangia, is exhibited by the repeated emergence of laterally-ciliate zoospores in *Dictynchus* sp., described by Weston (12), in *Achylya racemosa* Hildebrand, described by Höhnk (4), in *Phytophthora infestans* (Mont.) de Bary, described by Murphy (7), in *Pythium proliferum* de Bary, described

by Cornu (2), and in *Pythium diacarpum* Butler described by Butler (1). Still another type of repetitional development, in which the sporangiospore germinates, forming at the tip of the germ tube a new sporangium, which emits only one zoospore, occurs in *Pythium dictyosporum* Racib. (9) and in *Phytophthora* sp., associated with pink rot of potato (3).

Primary sporangia may produce tubes surmounted by secondary sporangia in certain species of *Phytophthora*. Two secondary sporangia are thus formed by *Peronospora cactorum* (Cohn et Leb.) Schroet. shown in Rosenbaum's<sup>1</sup> figure 16 (10). Another of his illustrations in the same figure shows that tertiary sporangia may arise. Proliferation of this type with production of a single secondary sporangium was noted in *P. infestans* by Jones, Giddings, and Lutman (5: Fig. 6) and in *P. phaseoli* Thaxter by Thaxter (11: Pl. 3, figs. 31-32). These species may, therefore, exhibit a proliferative development analogous with that herein noted in *Peronospora tabacina*.

Apparently, proliferation of the kind illustrated in figure 1, h, finds no counterpart among the related families of Phycomycetes. In the Peronosporaceae all of the protoplasmic content most commonly migrates from the sporangiophore into the sporangia when the sporangia form, leaving the sporangiophore empty. Presumably, differentiation does not occur during migration of the protoplasm into the sporangia, so that each sporangium is totipotent. Repetitional development of this type could therefore be anticipated to occur. Sufficient explanation of the function of the other types of proliferation would appear to require only the statement that the fundamental law of life of each organism is reproduction of its kind.

Höhnk (4) pointed out that there is a tendency from planetism to aplanetism among the Saprolegniaceae, correlated with progression from water forms toward land forms. Conceivably, polyplanetism, well known among the Saprolegniaceae, and proliferation may be parallel developments, and both may have phylogenetic significance.

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## RELATION OF STOMATA TO INFECTION OF TOBACCO LEAVES BY BACTERIUM TABACUM<sup>1</sup>

STEPHEN DIACHUN

(Accepted for publication October 23, 1939)

One of the common portals of entry for bacteria that cause leaf spots is the stoma (6, 9). It is believed that in natural field infections *Bacterium tabacum* Wolf and Foster usually invades tobacco leaves through stomata (1, 3, 5). When leaves are artificially inoculated by atomizing with a bacterial suspension the bacteria presumably enter through stomata. Clinton and McCormick (3) reported that when immature tobacco leaves were sprayed, infections rarely occurred, indicating that "entrance takes place through the stomates, which in these leaves are not so fully developed or liable to be open."

On several occasions, both in the field and in the greenhouse, when leaves were atomized with bacterial suspension late in the afternoon or on dull, cloudy days, only a very limited amount of infection resulted. Diachun and Valleau (4) have reported that stomata of leaves of greenhouse tobacco plants are usually wider open during the day than at night, and on sunny days than on very dull days. Subsequent field tests have shown that, in general, the results obtained on greenhouse plants are applicable also to field-grown plants.

It is the purpose of this paper to report results of experiments designed to determine the effect of stomatal opening at the time of inoculation on infection of tobacco leaves inoculated by atomizing with *Bacterium tabacum*.

### EXPERIMENTAL RESULTS

To determine whether the degree of stomatal opening, and, consequently, the time of inoculation, influences the amount of infection, the following tests were made. On January 7, 1939, 6 leaves of each of 3 vigorous white Burley plants were atomized by means of a de Vilbis atomizer with a 24-hour broth culture of *Bacterium tabacum* diluted with 10 parts of sterile water.

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

The nozzle of the atomizer was held 2 inches from the lower surface. Plant A was inoculated in the greenhouse at noon. Plant B was placed in a dark room at 9 a.m., inoculated in the dark room at noon, and kept there until 6 p.m. Plant C, which was in the greenhouse all day, was inoculated at 6:30 p.m., more than an hour after twilight. At the time of inoculation several pieces of epidermis were stripped from each plant for stomatal examination, using Lloyd's technique (8). To compare the stomatal opening of leaves on the 3 plants an index expressed as  $K^2$  was used. The value of K for plant A was .32; for plant B, .13; and for plant C, .14. That is, the stomata on plant A were more than twice as wide as those on plants B and C. By January 16, plant A was heavily infected. There were only 15 isolated wildfire spots on plant B, and on plant C only 4 such spots. This experiment was repeated 5 times with similar results: the stomata were open on plants inoculated in the greenhouse at noon and infection was severe; they were nearly closed on plants inoculated at night and in artificial darkness, and infection was limited (Fig. 1).

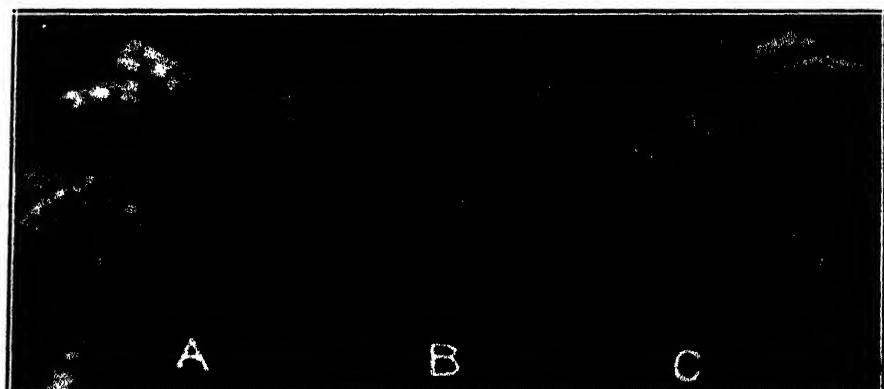


FIG. 1. Effect of stomatal opening on infection with *Bact. tabacum*. The leaves were atomized with a 24-hour broth culture diluted with 10 parts of sterile water. The nozzle of the atomizer was held 2 inches from the lower surface. Inoculated February 13, 1939, and photographed February 20. A. Inoculated in the greenhouse at 2:30 p.m. on a sunny day, stomata open,  $K = .25$ . B. Placed in dark room at 11:30 a.m., inoculated in dark room at 2:30 p.m., stomata nearly closed,  $K = .05$ . C. Inoculated in the greenhouse at 7 p.m., stomata nearly closed,  $K = .06$ .

Inoculations were made also in the field on several occasions when stomata were closed. Infection was always less severe when stomata were closed than when they were open. For example, on August 7, 1939, the left side of 2 leaves was inoculated at 9 p.m., when the stomata were nearly closed, K being .05. The right side of the same leaves was inoculated at 6:15 the next morning when the stomata were open, K being .25. Both sides were inoculated by atomizing with the atomizer 2 inches from the lower surface. By August 15 there was good infection on the right side and only a very few spots on the left side of each leaf. Again, on August 8, the

<sup>2</sup> K is the ratio of the average actual width to the average potential length of the opening of 20 measured stomata.

right side of a leaf of a dark tobacco plant was inoculated by atomizing at 4 p.m. The lower surface of the leaf was exposed to direct sunlight and the stomata were nearly closed.<sup>3</sup> The left side of the same leaf was inoculated at 9 the next morning, when the stomata were open. By August 15 there was good wildfire infection on the left side and but little on the right side of the leaf (Fig. 2).

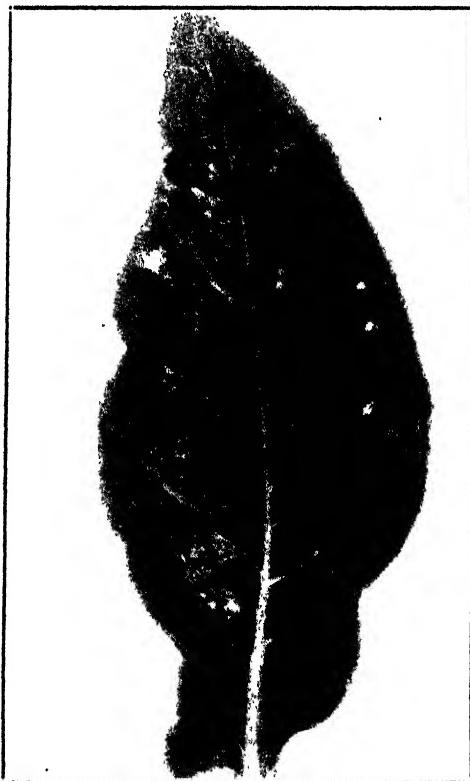


FIG. 2. Effect of stomatal opening on infection of a field-grown plant with *Bacillus tabaci*. Both sides of the leaf were atomized with the nozzle of the atomizer 2 inches from the lower surface. The right side was atomized at 4 p.m., August 8, when stomata were nearly closed. The left side was atomized at 9 a.m., August 9, when stomata were open. Photographed, August 21, 1939.

#### DISCUSSION

The results of the experiments reported here call attention to the fact that when leaves of tobacco, and perhaps other plants, are to be inoculated with bacteria by atomizing, the condition of the stomata must be considered. Erroneous conclusions about the resistance or susceptibility of plants being compared, or about the virulence of cultures under test may be reached unless care is taken to make all inoculations when stomata are known to be open. Leaves that are shaded, wilted, or turned up with the lower surface

<sup>3</sup> It was observed frequently that when leaves were turned up, so that the lower surface was exposed to the afternoon sun, the stomata were closed.

exposed to the sun, should be avoided, for observations have shown that stomata are likely to be closed under these circumstances.

It is generally believed that there is a close correlation between outbreaks of wildfire and stormy, rainy weather. Chapman and Anderson (1) have said, "It has been noted by all investigators of the disease and by tobacco growers that rapid spread invariably follows heavy rains. . . . These two agents (wind and rain) are undoubtedly the most potent of all factors involved in dissemination." Some workers have believed that heavy storms produce wounding, which facilitates invasion. J. Johnson and Fracker (7) reported that storms, especially beating rains, "have a very important relation to wildfire in that they favor infection to a high degree. The bacteria are often unable to infect leaves except through slightly wounded tissue such as may be produced by beating rain." More recently, Clayton (2) has expressed the belief that storms are important in connection with water-soaking. He feels that the occurrence of epidemic wildfire is "conditional on storms of sufficient severity and duration to produce and maintain water-soaked areas on the leaves." Unpublished observations of W. D. Valleau and E. M. Johnson indicate that, although outbreaks of wildfire are often associated with rainy, stormy weather, every storm does not contribute to infection and spread. From the experiments reported here it is concluded that atomizing tobacco leaves with *Bacterium tabacum* produces infection only if the stomata are open. The suggestion occurs that perhaps natural infection by bacteria carried in windblown rain may occur only when stomata are open. It has been observed that during some rain-storms stomata are open; during others, they are closed, depending perhaps on the light intensity. On July 6, 1939, at 3:30 p.m. there was a light shower; light was 800 foot-candles; stomata were open, K being .24. On July 20 it rained intermittently all morning; light was 600 foot-candles; stomata were open, K being .23. On July 28 it rained at 4 p.m.; stomata were open, K being .16. On July 29 it rained from 8 to 10 a.m.; light was 300 foot-candles; stomata were open, K being .25. However, on July 19, 1939, when it rained at 12:30 p.m., the light was only 100 foot-candles, and stomata were closed, K being .05. It cleared up in a short time; at 2 p.m. light was 2000 foot-candles, and stomata were open on some of the leaves. At 4:30 p.m. it rained again, light was less than 100 foot-candles, and stomata were closed. Although there was little wind with these rains, it is conceivable that high winds accompanying such rains could dash drops of water with bacteria against leaves with sufficient force to drive some of the water into the leaf if the stomata were open, while similar storms might not produce infection if the stomata were closed.

#### SUMMARY

The experiments reported here indicate that in the greenhouse or field the stomatal condition of leaves is a factor determining the amount of infection on leaves atomized with a suspension of *Bacterium tabacum*. During

the day the stomata are usually open, and atomizing produces heavy infection on tender leaves of vigorous plants. At night or in artificial darkness stomata are closed or nearly so, and leaves atomized with *Bacterium tabacum* develop only a limited amount of infection (a few open stomata were always observed, even on plants in the dark).

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#### ATTEMPTS TO ISOLATE CERATOSTOMELLA ULMI FROM STORED ELM WOOD

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(Accepted for publication Nov. 22, 1939)

*Ceratostomella ulmi* (Schwarz) Buisman, the fungus causing the Dutch elm disease, is known to survive the death of the host long enough to contaminate elm bark beetles emerging from infected dead wood. The present study was carried on in the hope of learning more about the longevity of *C. ulmi* in cut elm wood and some of the field conditions affecting it.

In late September, 1936, infected living branches 1 to 4 inches in diameter were cut from several American elms affected by the Dutch elm disease. The branches were cut into 611 one-foot lengths and divided into 3 approximately equal lots for storage under different field conditions. Each lot was so selected as to include about one-third of the sticks from each tree. Lot 1 was placed on the ground in deep forest shade. Lot 2 was laid on the grass in an area unshaded, except for weeds which grew up and were cut during the middle of the summer. Lot 3 was placed on a rack 18 inches above the ground, where the sticks were in direct sunshine for the greater part of the day. All bark was removed from half of the pieces in each lot and peeled sticks were placed in groups beside the non-peeled in each of the three environments. The side of each diseased stick, showing at the cut ends the most severe discoloration indicative of the Dutch elm disease, was marked

for subsequent culturing. The pieces in each lot were laid so that the marked side of every other one was on top, while that of the alternate sticks was placed downward. Unfortunately, there was not time to attempt to isolate the fungus from each stick at the outset of the experiment. The distribution of the diseased wood is summarized in table 1.

TABLE 1.—*Recovery of Ceratostomella ulmi from diseased elm wood stored variously*

Environment of stored sticks, position of discoloration cultured, and condition of sticks	Number of diseased sticks	Percentage of sticks cultured yielding <i>C. ulmi</i> after—	
		4 months	20 months
<b>Woods:</b>			
Discoloration down—			
Bark off . . . . .	68	73	2
Bark on . . . . .	69	95	23
Discoloration up—			
Bark off . . . . .	63	29	0
Bark on . . . . .	62	70	8
<b>GRASS:</b>			
Discoloration down—			
Bark off . . . . .	67	39	0
Bark on . . . . .	63	62	2
Discoloration up—			
Bark off . . . . .	62	17	0
Bark on . . . . .	51	43	0
<b>RACK:</b>			
Discoloration down—			
Bark off . . . . .	59	35	4
Bark on . . . . .	66	86	2
Discoloration up—			
Bark off . . . . .	64	33	0
Bark on . . . . .	67	87	0

Fifty similar pieces of non-diseased wood, 25 peeled and 25 non-peeled, were stored in each of the 3 environments as checks.

In January, 1937, 4 months after the experiment was begun, an attempt was made to isolate *Ceratostomella ulmi* from one-third of the total number of sticks so chosen as to represent each condition of storage. After attempted isolation, the sticks were returned to their previous environments. In May, 1938, 20 months after the initiation of the experiment, an attempt was made to isolate the fungus from all the sticks. The experiment was then concluded. On both dates isolation was attempted in two ways by placing chips cut from the wood and flooded briefly with hydrogen peroxide (a) into Petri dishes containing potato-sucrose agar, and (b) into Petri-dish moist chambers, where they were incubated for 3 weeks at approximately 60° F. If, during incubation, coremia developed on the chips, some of their spore-containing exudate was transferred to potato-sucrose agar to determine with certainty whether the coremia were those of *C. ulmi* or of closely related species that form somewhat similar coremia.

Table 1 shows the percentages of the total numbers of cultured diseased sticks stored in each manner from which *Ceratostomella ulmi* was recovered after 4 and after 20 months. None of the check sticks yielded *C. ulmi* when cultured. While the mathematical significance of the data in table 1 has not been tested, inspection of them leads to the following conclusions: (1) With the passage of time, *C. ulmi* was recoverable from fewer sticks. After four months' storage only a part of the sticks cultured which were stored under each condition yielded the fungus. After 20 months, *C. ulmi* was recoverable from only a small percentage of all sticks, except those stored in the woods with the bark on and the side marked for culturing oriented downward. Twenty-three per cent of these still yielded *C. ulmi* when cultured after 20 months' storage. (2) Peeled sticks yielded *C. ulmi* in consistently lower percentages than non-peeled sticks after 4 months under all storage conditions. After 20 months the differences between peeled and non-peeled sticks were slight or negligible, except for those sticks stored in the woods with the side marked for culturing oriented downward. Under these conditions only 2 per cent of the peeled sticks yielded *C. ulmi*, while the fungus was recovered from 23 per cent of the non-peeled sticks. (3) The effects of the place of storage and orientation of the side marked for culturing were shown with less consistency and were in many cases small or negligible.

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#### PHYTOPATHOLOGICAL NOTES

*Selenized Soil as a Control for Aphids and Red Spiders on Sorghum in the Greenhouse.*—Greenhouse studies on sorghum diseases are frequently complicated by infestations of aphids, *Aphis maidis* (Fitch), and red spiders, *Tetranychus telarius* (L.), to both of which pests sorghum is very susceptible. The effects of aphid and red-spider infestation on the plants frequently mask the symptoms of the disease being studied. Frequent fumigations and sprayings necessary for the control of these pests are laborious, expensive, and sometimes injurious to the plants.

Hurd-Karrer and Poos<sup>1</sup> in 1936 controlled aphids on wheat, oats, rye, and barley in the greenhouse by adding 10 p.p.m. selenium as sodium selenate to the soil; both aphids and red spiders were controlled on wheat plants grown in nutrient solutions containing from 1 to 3 p.p.m. of selenium. Morris *et al.*<sup>2</sup> reported control of red spiders on corn grown in nutrient solutions to which 1 p.p.m. selenium was added weekly.

In September, 1939, the writer grew sorghum in 4 sections of a green-

<sup>1</sup> Hurd-Karrer, A. M., and F. W. Poos. Toxicity of selenium-containing plants to aphids. *Science* 84: 252. 1936.

<sup>2</sup> Morris, V. H., C. R. Neiswander, and J. D. Sayre. Toxicity of selenium-containing plants to red spiders as a means of control. (Paper presented before the 16th annual meeting of the American Society of Plant Physiologists December 28-30, 1939, at Columbus, Ohio.)

house bench filled with Keyport clay loam to which had been added 0, 5, 10, and 15 p.p.m., respectively, of selenium in the form of sodium selenate, which was thoroughly mixed into the whole mass of soil. Emergence was not affected by the selenium, but, after 2 weeks, the average height of the plants was reduced by the 3 selenium concentrations 19, 43, and 50 per cent, respectively, compared with that of the control without selenium. All attempts to infest these plants with aphids or red spiders were futile. The controls, however, became severely infested and were badly damaged. In a second planting in these same sections 3 months later, a reduction of 5 to 25 per cent was observed in the height of the plants in the selenized soil, and



FIG. 1. Control of aphids and red spiders on Dwarf Yellow milo: Left, badly infested plants grown without selenium; right, noninfested plants grown in soil containing 2 p.p.m. of selenium.

the freedom from insect infestation persisted, although the controls became heavily infested.

Similar plantings also were made in soil containing 2, 3, and 4 p.p.m. of selenium. Measurements made 4 weeks after emergence showed no reduction in height of plants in the selenized soil. The controls, grown without selenium, soon became heavily infested with both aphids and red spiders. In the soil containing 2 p.p.m. selenium, red-spider infestation was observed after 8 weeks, but no injury was evident. The plants were green and healthy, while the controls without selenium were stunted and badly discolored (Fig. 1). After 14 weeks the plants grown in soil containing 2 p.p.m. selenium showed some evidence of red-spider reproduction and injury. Those grown in soil containing 3 p.p.m. selenium showed some infestation but no apparent injury, while those in soil containing 4 p.p.m. selenium remained free from red spiders and aphids. The Leoti sorgo grown in the selenized soil had formed normal heads and seed after 14 weeks, while, in the control soil, the plants were stunted and had produced no heads.

The length of time that one application of selenium to the soil will provide protection against aphid and red-spider infestation, and the optimum time, manner, and rate of applying additional selenium remain to be determined. It remains to be determined also what effect, if any, the selenium may have on the development of the several diseases of sorghum. It was noted that sorghum root rot developed as abundantly in the selenized as in the nonselenized soil, which indicated that the selenium did not inhibit the development of this disease.

It should be emphasized that selenium is extremely poisonous to man and other warm-blooded animals and, therefore, under no circumstances should it be used as here described in connection with plants intended for other than experimental purposes.—R. W. LEUKEL, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

*Sectoring in Colonies of Aplanobacter stewarti.*—For the purpose of stimulating dissociation, 5 single-cell cultures of *Aplanobacter stewarti* have been used during the past year in daily serial transfers to different broth combinations and reactions, and daily platings have been made on beef-peptone agar. These 5 cultures produce wilt lesions on corn and develop on beef-peptone agar as typical yellow colonies, but they differ more or less in pathogenicity and in physiological reactions. After alternating periods of daily transfer and of ageing, no dissociation was observed on beef agar; but, on potato-dextrose-agar slants of culture No. 3b6, pure white areas developed in the yellow growth. Platings on potato-dextrose agar from the same broth cultures gave numbers of colonies nearly all of which were sectoring for color and possibly other characters. On 1 plate there were 3 colonies, 2 of which had lighter yellow sectors and 1 with a pure white

sector (Fig. 1). On the second plate were 50 colonies, a few pure yellow and a few pure white without sectors; but most of the colonies had 1 or more sectors of different shades of yellow or white. Three colonies on the first plate and 20 on the second were selected for further study. Transfers were made from each colony and from 1 or more sectors in that colony, making 53 transfers. Each of these transfers was replated for purity on potato-dextrose and beef-extract agar.

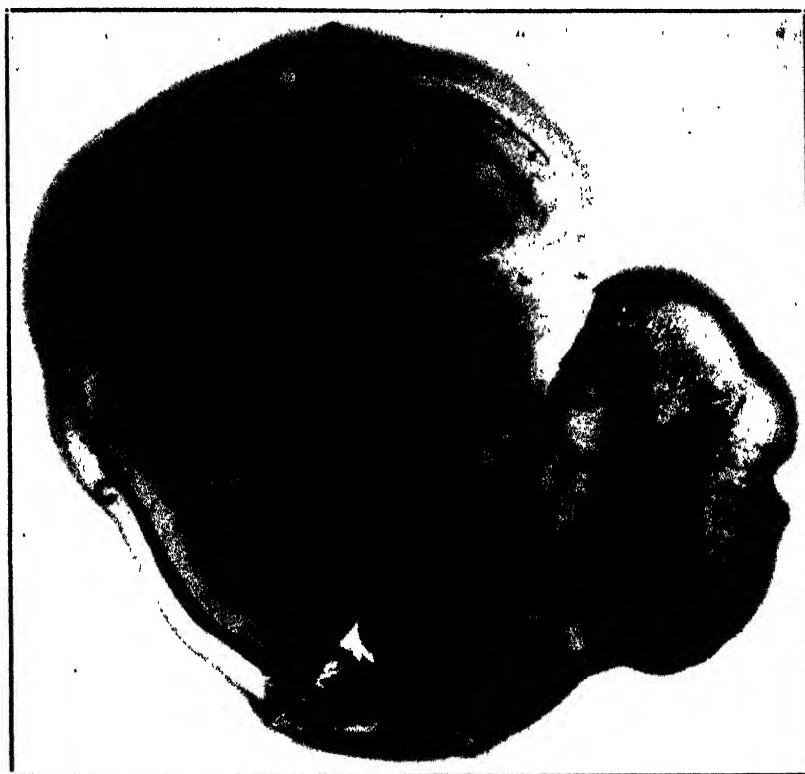


FIG. 1. Sectoring colony of *Aplanobacter stewarti* 38A3b6 (single-cell isolation). Plate I, colony 1. On potato-dextrose agar. Yellow colony with pure white sector. Photographed on April 17, 1939. A. Yellow colony. B. Pure white sector. C. White streaks.  $\times 4$ .

From 2 pure white colonies without sectors, only white colonies were obtained. From 9 transfers from white colonies without sectors, white colonies with yellow sectors and white sectors in yellow colonies, all but 2 gave pure white cultures. One transfer from a white colony without any apparent sectors gave a few yellow in many white colonies. One transfer from a white sector in a yellow colony gave 1 yellow in many white colonies. Platings from 4 light yellow (almost white) colonies or light yellow (almost white) sectors gave either light yellow colonies, white and yellow, or yellow colonies. Four yellow colonies, which had white sectors, gave only yellow colonies. Thirty platings from yellow sectors in light yellow colonies, yel-

low colonies, with light yellow sectors, yellow sectors in yellow colonies, and yellow colonies with yellow sectors gave white colonies in only 2 plates. Pure yellow colonies without sectors gave only yellow colonies. Thus some white colonies remained white, most yellow remained yellow, some light yellow segregated into white and yellow, while others remained light yellow or resumed the typical yellow of this species.

When inoculated into corn and reisolated from wilt lesions, white cultures remained white and yellow came out yellow.

The parent single-cell culture No. 3b6 is only weakly virulent, while the other 4 single-cell cultures are virulent. In 4 tests for pathogenicity with the 53 transfers of white and yellow colonies or sectors, there was no evidence that any of these cultures from sectoring colonies of 3b6 was more virulent than the original culture.

The 3b6 parent culture also differs from the other 4 single-cell cultures in that the reaction in litmus milk is sufficiently alkaline to produce a blue color, which darkens as the culture ages. All of the other 4 cultures are slightly acid in litmus milk, producing a pink color without curdling the milk. This is the typical reaction for *Aplanobacter stewarti*. The 53 transfers from white and yellow colonies and sectors when grown in litmus milk all produce the blue color typical of the parent strain 3b6. Variation in this instance appears to be for color only, the organism remaining stable as far as other characters tested are concerned.—CHARLOTTE ELLIOTT and ALICE L. ROBERT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

**A Specimen-envelope Folder.**—Many laboratories and herbariums use specimen envelopes. When these are made by the usual technician or laboratory assistant they may vary considerably in size and shape.

A mechanical device that gives uniformity of envelope has been in use in our laboratory and is here illustrated and described. It is suited for making envelopes  $3\frac{1}{4} \times 5\frac{1}{2}$  in. from  $8 \times 9$  in. paper. The same general pattern can be modified to make envelopes of any desired size. The apparatus is of simple and easy construction and was designed by the late D. S. Giddings of our laboratory.

The device consists of a firm back,  $10 \times 11$  in., of fiber board or thin wood on which guide blocks are to be fastened, and two iron plate folders. A white covering of paper or thin cardboard is to be fastened to the back. It is best first to make a drawing showing where the wood blocks are to be fastened.

To this flat back, thin blocks of wood are nailed. In our folding device these blocks were cut from the rounded ends of 12-in. pot labels  $1\frac{1}{2}$  in. wide. Three different lengths of these blocks are required: 4 blocks  $2\frac{1}{2}$  inches long (Fig. 1, A); 2 blocks  $2\frac{3}{8}$  inches long (Fig. 1, B); 2 blocks  $1\frac{1}{2}$  inches long (Fig. 1, C).

In placing the blocks A, B, and C, extra space of  $\frac{1}{16}$  in. should be allowed

for all distances between blocks. This will provide room for operating the folders and placing the paper.

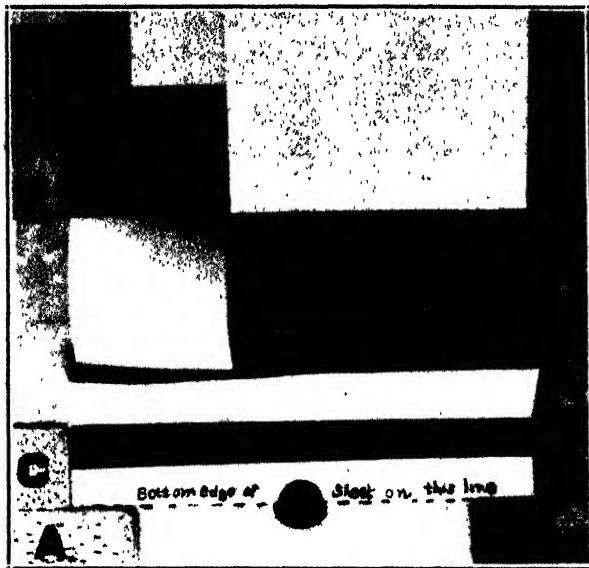


FIG. 1. General view of specimen envelope folder: A, B, and C represent blocks and show their relation to the iron plate folder, to the paper, and to the first folds of the envelope. The upper fold is cut to show the position of the upper blocks, A and B.



FIG. ... show blocks in position on back board and their relation to second iron plate folder and envelope, one end of which is already folded.

The two rectangular iron folder plates are shown in place in figures 1 and 2. They are made from thin galvanized iron and are each provided with a handle. The long edges of the plates are ground on the upper side to form an angled edge to give the folded paper a sharp crease. They are each 10 in. long, the same size as the width of the apparatus. The narrow folder is 3 $\frac{1}{2}$  in. wide, the width of the envelope to be made. Figure 1 shows

this folder in position where it is to assist in the making of 2 folds in the paper. The lower fold is already made. The top of one part of the envelope paper is in position unfolded (Fig. 1), while, at left, the portion is folded in the manner that the uncut sheet would be in practice. The second or wide plate folder for making the 2 end folds of the finished envelope, is 5 $\frac{1}{4}$  in. wide and equals the length of the folded envelope. Figure 2 shows it in position and one end of envelope already folded. It is more convenient to make folds in a number of envelopes with the first folder before completing the end folds with the second.—CLAYTON O. SMITH, University of California, Citrus Experiment Station, Riverside, California.

*The Chilean Tomato, Lycopersicon chilense, Found Resistant to Curly Top.*—In the February issue of *Phytopathology* for 1939, F. O. Holmes<sup>1</sup> published a note on the Chilean tomato, *Lycopersicon chilense* Dun., as a possible source of disease resistance. Through his kindness cuttings of this species were obtained for the purpose of testing their reaction to curly top. They grew well, and it was possible to get many plants from them.

The first test was conducted in the University greenhouse at Moscow, Idaho. Beet leaf hoppers (*Eutettix tenellus* Baker), which had been fed on curly-top sugar beets, were placed on 2 *Lycopersicon chilense* plants and on 2 tomato plants of the variety Earliana. Cages were placed over the plants to keep the insects confined to them. The leaf hoppers were left for 48 hours before their removal. The two Earliana tomato plants became severely diseased with curly top and died, whereas the *L. chilense* plants remained healthy, and no symptoms of the disease were observed.

The second test was conducted in the field at Buhl, Idaho, where curly top is usually severe every year. Small *Lycopersicon chilense* plants, started in the greenhouse from cuttings, were transplanted to the field. After they had established themselves beet leaf hoppers were caged on 3 of the plants. These leaf hoppers had been fed on diseased sugar beets prior to placing them on the *L. chilense* plants. The cages were removed after several days. No symptoms of curly top developed, and the plants grew luxuriantly throughout the season. No natural infection occurred on them, as did on the common tomatoes growing in the same field.

It appears that *Lycopersicon chilense* is resistant to curly top, and an attempt is being made to incorporate this resistance in several varieties of the common tomato.—WALTER J. VIRGIN, University of Idaho, Moscow, Idaho.

<sup>1</sup> Holmes, F. O. The Chilean tomato, *Lycopersicon chilense*, as a possible source of disease resistance. *Phytopath.* 29: 215-216. 1939.

# EVIDENCE FOR THE IDENTITY OF THE YELLOW-SPOT VIRUS WITH THE SPOTTED-WILT VIRUS: EXPERIMENTS WITH THE VECTOR, THrips tabaci<sup>1,2</sup>

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In June, 1937, an outbreak of a tomato disease, indistinguishable from spotted wilt, was observed by G. K. Parris of the Hawaii Agricultural Experiment Station, in a small field on the island of Oahu, Hawaii (31). To identify the causal virus and to obtain evidence for the coidentity of the tomato spotted-wilt virus with the pineapple yellow-spot virus,<sup>3</sup> which has long been established in Hawaii, a project was started jointly by the Hawaii Agricultural Experiment Station and the Pineapple Experiment Station. Parris undertook symptomatological studies and mechanical transmission tests, and his data are presented in a concurrent paper (32). The writer conducted experiments on insect transmission of Y.S. virus to tomato and various other plants. Data are presented here showing that Y.S. virus is the causal agent of the tomato disease and that the host range and symptoms of the two viruses are similar.

## REVIEW OF LITERATURE

Brittlebank (9) recorded the first appearance, in 1915, of tomato spotted wilt in Victoria, Australia. Pittman (33), Samuel *et al.* (37), and Bald *et al.* (5) in 1927, 1930, and 1931 demonstrated that the virus is transmissible by *Thrips tabaci* Lind. and *Frankliniella lycopersici* And. (3) and also by the rubbing method of mechanical inoculation with expressed plant juice. The specific relationships between the virus and vectors, longevity, and heat resistance of the virus *in vitro*, and many host plants were reported.

Smith (41, 42), in 1931 and 1932, discovered the same virus in England, where *Thrips tabaci* is the sole vector, and reported on the filterability of the virus and its host plants. Furthermore, Moore (30) in 1933 discussed the possible coidentity of Krommek disease in South Africa, which is transmissible by *Frankliniella* sp., with spotted wilt. Her final conclusion on this and reference to the specific vectors, *F. schultzei* (Trybom) and *T. tabaci*,<sup>4</sup> by E. E. Anderssen, both of which will be published shortly, were cited by Carter (13). Since 1929 the same virus has been discovered in California, Wisconsin, and Ontario (7, 16, 22, 40). Gardner *et al.* (18, 19, 20) in 1934, 1935, and 1937 reported from California its transmission by *T. tabaci* and

<sup>1</sup> Published with approval of the Director as Technical Paper No. 129 of the Pineapple Experiment Station, University of Hawaii.

<sup>2</sup> This investigation was conducted under the general direction of Dr. Walter Carter.

<sup>3</sup> Hereafter the spotted-wilt virus will be referred to as S.W. virus; the yellow-spot virus as Y.S. virus.

<sup>4</sup> The addition of *T. tabaci* is, with his permission, directly cited from an oral communication.

*Frankliniella moultoni* Hood<sup>5</sup> together with a long list of host plants. McWhorter and Milbrath (27, 29) stated that the virus of tomato tipblight, known in southern Oregon since 1931, is very closely related but distinct from S.W. virus, although it is transmitted by *T. tabaci* also.<sup>6</sup>

In addition to the above references, there are more than 100 papers dealing with economic significance, new invasion or distribution, and host range in Australia, Europe, Africa, and North America. India is also included in the distribution data (26). The known host range is very large and a total of 101 host plants was enumerated by Smith (45) in his latest review. This number of hosts of a single virus is exceeded only by those of curly-top, aster yellows, tobacco mosaic, and cucumber mosaic. Many other valuable contributions have been recently published concerning chemical and physical properties and serological response of the virus, epidemiology of the disease, and environmental effects on symptom manifestation.

Linford (25) in 1932 published an account of pineapple yellow spot, which had been known in Hawaii since 1926. The virus was demonstrated to be transmissible by *T. tabaci*. The specific relationships between the virus and vector were found similar to those of S.W. virus. Preliminary experiments on mechanical transmission to pineapple were reported as being negative. Later, Carter (11) reported a single successful transmission by the needle puncture method. In addition to pineapple and *Emilia sonchifolia* DC.,<sup>7</sup> a major weed host of the virus, Linford (24) added pea to the host list. He also prepared in 1931 a manuscript<sup>8</sup>, which still remains unpublished, but citation of which was granted, on the host range and symptoms. In the manuscript he mentioned that the virus was experimentally transmitted by *T. tabaci* to 17 other species of plants (his complete list has been cited in another paper (35)), including pepper, tomato, tobacco, and eggplant. Symptoms on some plants were described in detail and on others, very briefly Kitamura<sup>9</sup> mentioned 6 susceptible plants including one addition to the host list.

Linford recognized the close similarity between Y.S. and S.W. viruses with respect to the vector and the specific relationships between the vector and virus (25), but only a partial similarity with respect to the symptoms

<sup>5</sup> Inclusion of *F. occidentalis* Perg. as the vector (17), according to a private correspondence from Dr. S. F. Bailey, University of California, is a matter of different view on classification of the species. Permission was granted to cite his unpublished data.

<sup>6</sup> *T. tabaci* was reported in a recent paper (Chamberlain, E. E., and G. G. Taylor. N. Z. Jour. Sci. and Tech. 20: 133A-142A. 1938) to be the vector of S.W. virus in New Zealand.

Dr. A. S. Costa, Instituto Agronomico do Estado de S. Paulo, Campinas, Brasil, recently informed the writer that the virus of "Vira-cabeça" of tobacco and tomato, widely distributed in Brasil, has been proven to be identical with S.W. virus; that the vector is a species of *Frankliniella*, possibly *F. paucispinosa* Moulton, which is also known to be the vector of "Corcova" of tobacco and tomato in Argentina. (Fawcett, G. L. Est. Exp. Agr. Tucuman Cir. 60. 1938). Identity of "Corcova" with "Vira-cabeça" and spotted wilt has not been reported.

<sup>7</sup> Hereafter *Emilia sonchifolia* DC. will be referred to as *Emilia*.

<sup>8</sup> Linford, M. B. Some hosts and symptoms of pineapple yellow spot.

<sup>9</sup> Kitamura, F. T. The influence of host sequence on the efficiency of *Thrips tabaci* Lind. as a vector of the yellow-spot virus of pineapple. Thesis, Univ. of Hawaii. 1936.

and host range.<sup>10</sup> However, he was unable to come to any conclusion as to their coidentity from his data. Smith (42, 45) speculated on the probable identity of the two viruses. Although their coidentity has thus been long postulated, it has still remained to be verified with further proofs. Recently, the following new data further suggesting their coidentity have been reported. Gardner *et al.* (19) and, later, Whipple (49) reported transmissions of S.W. virus to *Emilia*, and Whipple stated that the symptoms were almost identical with those of Y.S. virus. Linford (24) stated that the symptoms of Y.S. virus on pea were similar to streak in the continental United States, and, later, Whipple (49) and Snyder *et al.* (46) transmitted S.W. virus to garden and sweet peas and concluded that this virus is a causal agent of streak. It is induced from the above that the symptoms of the two viruses on this host must be similar. Lewcock (23) and Carter (13) observed a pineapple disease identical with yellow spot in Queensland and South Africa, where S.W. virus was well established. Although neither of them presented any experimental data, there is little doubt that the causal agent is S.W. virus.

Finally, Parris (32) demonstrated that Y.S. virus was readily transmitted to tomato, *Emilia*, and potato by the carborundum method of mechanical inoculation, and this first accomplishment of mass mechanical transmission contributed an important direct evidence for the coidentity of the two viruses. He concluded that the tomato disease observed in Hawaii is caused by Y.S. virus.

#### FIELD OBSERVATIONS

*Emilia* plants were found growing abundantly in the tomato field, where the outbreak of the disease was observed. Random samples of these *Emilia* plants showed very high populations of *Thrips tabaci* (6.81 per plant) and incidence of virus infection (79.8 per cent) among them, whereas populations of *T. tabaci* on tomatoes were negligible (0.23 per sample of twigs collected). The symptoms on tomatoes were indistinguishable from those of spotted wilt and the symptoms on *Emilia* from those of yellow spot.

Subsequently, the field *Thrips tabaci* from these *Emilia* samples were transferred to 12 each of noninfected *Emilia* and pineapple seedlings. Ten each of both plants were infected, showing the presence of a very high percentage of viruliferous insects in the field. The symptoms appearing on *Emilia* and pineapple were identical with those of yellow spot. These field observations and preliminary experiments suggested that the virus present in the tomato field was Y.S. virus, that the infection of tomatoes was also due to the same virus, and that the symptoms of Y.S. virus on tomato were similar to those of S.W. virus.

#### MATERIALS AND METHODS

*Insects.* Noninfective stock colonies of *Thrips tabaci* were established from adults collected on field onions which are nonsusceptible to Y.S. virus,<sup>11</sup>

<sup>10</sup> See footnote 8.

<sup>11</sup> See footnote 9.

and were maintained on *Emilia* or onions throughout the experiments. The noninfectiveness of such colonies was frequently checked.

**Virus.** *Emilia* infected in the field by Y.S. virus was taken as the original source of the virus. The infective colonies of thrips were established on such plants by transferring insects from the noninfective colonies. Infected *Emilia* and tomatoes collected in the tomato field, where the outbreak of the disease was observed, were also employed.

**Plants.** *Emilia* was constantly used as the indicator plant, and pineapple seedlings also were used in the experiments with tomato. Tomato and 21 other species of plants were used in the host range experiments. The variety of tomato used throughout the experiments, excepting those specified in the varietal experiments, was Marglobe.

Noninfected plants were grown from seeds, except *Emilia*, which was freely propagated by cuttings, and were used while they were young. They were planted singly in tin cans and covered individually by various types of insect-proof cages that confined the whole plant. A tall cylindrical celluloid cage, 3 by 18 inches, with 6 large cloth-covered windows, was found satisfactory for young tomato seedlings. The plants were kept in a greenhouse with open sides.

**Methods.** Methods of handling the vector differed from those employed by former workers (25, 37, 42). Insects to be transferred were shaken off from source plants onto black paper and sucked into medium-size reservoir vials, from which random individuals were then sucked into small vials, 15 by 25 mm., that were inserted directly into the cages containing the test plant. In most cases 5 insects were transferred per plant. For localized feeding, the felt-cage method (34) was employed. These operations were performed in small laboratory rooms, which were thoroughly sprayed after every unit of transfer. Other routine precautions were taken to avoid confusion of insects from different sources.

It is known that the virus is not retained through the egg stage; the adults are unable to become infective *de novo*; and the incubation period within the vector usually lasts until after the insect's emergence (5, 25, 42). Therefore, for acquiring or recovering the virus from infected source plants, noninfective young larvae were transferred and allowed to feed on such plants during their entire larval period. For transmitting the virus, freshly emerged adults from infected source plants were transferred to test plants.

#### TRANSMISSION OF THE YELLOW SPOT VIRUS TO TOMATO

The first experiment was to produce the symptoms of Y.S. virus on tomato. Sources of the virus were twofold, one from the yellow-spot-infected *Emilia*, and the other from the infected *Emilia* collected in the tomato field where the outbreak of the disease was observed. Young larvae from a noninfective stock colony were transferred to these plants, and adults emerging from these respective colonies were transferred to *Emilia*, pineapple, and tomato test plants (Table 1). Tomato, even when young, was found to be an unsuitable

TABLE 1.—*Transmission of the virus, by Thrips tabaci, from yellow-spot-infected Emilia and infected Emilia collected in the tomato field where the outbreak of the disease was observed*

Source of virus	Test plants	No. of test plants			
		From Emilia to			Transmission back to Emilia from tomato
		Emilia	Pineapple	Tomato	
Yellow-spot- infected <i>Emilia</i>	Tested Infected	10 6	10 2	10 5	40 1
Infected <i>Emilia</i> from the tomato field	Tested Infected	10 10	10 9	10 7	51 16

food plant for *Thrips tabaci* under experimental conditions.

Cross transmission tests back to *Emilia* were all positive (Table 1). Freshly emerged adults from the colonies established on the experimentally infected tomatoes were transferred to *Emilia* test plants. The lower percentage of infection in the yellow-spot-infected *Emilia* group may be due to the aging of the infected source plants.

The symptoms produced by the virus from the 2 different sources were found to be indistinguishable on *Emilia*, pineapple, and tomato. This clearly indicates that the virus present among *Emilia* in the tomato field is none other than Y.S. virus, confirming the data of the preliminary experiment. The symptoms produced by Y.S. virus on tomato were found to be identical with those on the field-infected tomato and also with those of spotted wilt.

In addition to the above experimental infections, 70 out of 263 tomatoes of various varieties also were infected. The varieties used besides Marglobe were Break O'Day, Burbank, First Early, Globe, Ponderosa, Pritchard, Red Plum, Rutgers, Stone, and currant tomato (*Lycopersicum pimpinellifolium* Dunal). It was observed that all varieties were susceptible and that their symptoms did not vary significantly.

#### SYMPTOMS ON TOMATO

Symptoms on field-infected tomatoes and on plants experimentally infected by mechanical inoculation were fully described by Parris (32). Therefore, characteristic points observed by the writer on plants experimentally infected by means of the vector are briefly given as follows:

Primary lesions were not observed. Downward curling of leaves and inward rolling of leaflets appear first. Bronzing (Fig. 1, A)—round or irregularly shaped spots, single or concentric rings, zonation, network along veinlets, midrib or lateral vein banding, or large blotching—appears suddenly on young leaves and rapidly advances to dark grayish-brown necrosis with or without zonation (Fig. 1, B, C, D, E). Diffused chlorosis appears along margin of necrosis. Affected leaflets or leaves wither and drop with the advancement of necrosis on lanninas and petioles. Bronzing, then necrosis in

a streak form appears on stems, especially at or near terminals causing an appearance of die-back (Fig. 1, F). Stem necrosis sometimes kills plants if infected when young. Growth is stunted but is later restored and lateral shoots appear. On these aged plants, leaf distortion and yellowish mosaic mottling appear together with occasional bronzing and necrotic symptoms. Concentric rings of water-soaked sub-epidermal tissue or of dark purplish-brown superficial pigmentation appear on green fruits, and concentric rings or diffused large round spots of orange yellow on a red ground on ripe fruits. When numerous, these ring spots coalesce. Some fruits on apparently infected plants fail to show any symptoms.

The infrequency of bronzing as a symptom of yellow spot compared with spotted wilt, first observed by Linford<sup>12</sup> and again by Parris (32) and the writer, seems to be due to the rapid advancement of necrosis over bronzed areas. The writer, however, is convinced that this difference can be entirely



FIG. 1. Symptoms produced by the yellow-spot virus on tomato, experimentally transmitted by *Thrips tabaci*. A. Bronzing. B. Necrotic spot with concentric zonation. C-D. Necrotic midrib banding, blotching, and round spots with zonation. E. Necrotic round spots and rings, and veinlet necrosis. F. Necrosis on the petiole and stem at the tip.

attributed to the different climatic conditions under which the transmission occurred, but not to any difference between the two viruses. This opinion is based on the known facts that the symptoms of S.W. virus are variable under different climatic conditions (8, 18, 19, 30, 37, 38).

#### RECOVERY OF THE VIRUS FROM FIELD-INFECTED TOMATO

The next experiment was to recover the virus from field-infected tomatoes. Young larvae from noninfective stock colonies were transferred to the infected green or ripe fruits and to the twigs bearing fresh or aged symptoms, all of which were kept in large test tubes. The twigs bearing fresh symptoms from plants grafted with field-infected scions also were employed as source materials. Adults that emerged from these source materials were transferred to *Emilia*, pineapple, and tomato test plants (Table 2). A very high mortality of larvae on twigs of grown plants was observed. Cross transmission tests, with a similar procedure to that of the foregoing experiment, from the experi-

<sup>12</sup> See footnote 8.

mentally infected *Emilia* to tomato and also to *Emilia* and pineapple test plants were all positive (Table 2).

TABLE 2.—*Transmission of the virus, by Thrips tabaci, from field-infected tomato*

Source of virus	Test plants	No. of test plants					
		From tomato to			Transmission back from <i>Emilia</i> to		
		<i>Emilia</i>	Pineapple	Tomato	<i>Emilia</i>	Pineapple	Tomato
Fruits:	Tested	54	39	72	31	16	15
	Infected	14	6	3	25	15	8
Ripe	Tested	10	10	10			
	Infected	0	0	0			
Twigs:	Tested	10			15	5	5
	Infected	3			12	4	3
	Tested	39	33	52			
	Infected	0	0	0			
Twigs from plants grafted with field- infected scions	Tested	57	35	35			
	Infected	11	8	1			

Similar to the findings with S.W. virus (1, 5), the virus was not recovered from ripe fruits and twigs with aged symptoms. The comparatively lower percentage of infection in this experiment seems to be attributable to unsuitableness of source plants for insect feeding and to loss of potency of the virus under certain conditions of the source plants. The symptoms produced on *Emilia*, pineapple, and tomato were indistinguishable from those of the experimentally infected plants by Y.S. virus in the foregoing experiment. These data on the recovery together with those on the transmission of Y.S. virus are adequate enough to conclude that the causal agent of the tomato disease in Hawaii was the long-established Y.S. virus, and not any other newly imported agent. Furthermore, it is definite that the symptoms of yellow spot on tomato are identical with those of spotted wilt.

#### HOST RANGE

Host range and symptom expression of a virus are taken as two of the criteria for its identification or classification. Here, the symptoms of Y.S. virus produced on various plants were directly compared with the published descriptions of the symptoms of S.W. virus. Twenty-one species of plants were tested, all but 5 of them crop plants. Nearly all of the crop plants of known status for the susceptibility to S.W. virus are included.

*Experimental Procedures.* Infective adults were transferred to test plants: The first lot, by freeing 5 adults directly into the cage; the second lot, by confining a single adult in the localized feeding felt cage (34), fastened on a young leaf for the purpose of detecting primary lesions; the third lot, by freeing 5 adults directly into the cages containing *Emilia* check plants in order to ascertain the infectivity of the insects (mostly, 10 plants for a single

unit of transfer). As a check for differentiating mechanical feeding injuries and symptoms, another lot of equal numbers of plants was similarly fed on by noninfective adults with both the free and localized feeding methods; noninfested check plants were kept in large numbers.

Recovery tests of the virus from the experimentally infected plants always were made, except on a very few plants on which larvae could not survive. Adults that emerged from the colonies established on the infected plants were retransferred to *Emilia* test plants. To ascertain the presence of masked symptoms or nonsusceptibility of plants, a similar transferring was made also from nonsymptom-bearing plants that, apparently, had been fed upon by infective insects. The carborundum method of mechanical inoculation was substituted in one case.

Numerical data are presented in table 3, with the combined figures of the infection by the free and localized feedings. The figures on the *Emilia* check lots, the noninfective adult check lots, and the recovery tests from infection-escaped plants of suspects are omitted. The degree of susceptibility for infection and suitability of the plants for insect feedings are summarized in the table.

**Symptoms.** The symptoms produced by Y.S. virus on broad bean, celery, potato, tobacco, *Nicotiana glutinosa* L., *Datura stramonium* L., petunia, and lettuce were found to be identical with those of S.W. virus. The characteristic symptoms on the respective plants are summarized in table 4. The susceptibility of *Datura stramonium* to Y.S. virus was first reported by Kitamura,<sup>13</sup> but the symptoms he recognized did not agree with those of S.W. virus. Contrary to his findings, however, the symptoms observed in the present experiment were found to be fully identical.

Descriptions of the symptoms of spotted wilt on spinach, chicory, and endive, all susceptible (20), have not been heretofore published, making it impossible to compare with the symptoms of yellow spot; descriptions of the symptoms on eggplant and bell pepper are extremely brief (5, 41, 42) and critical comparisons also are impossible. However, the symptoms on the latter two produced by Y.S. virus were found to be of the same type as those described of S.W. virus. The symptoms on these 5 species of plants produced by Y.S. virus are described as follows.

**Spinach.** Primary lesions were not observed. Systemic symptoms appearing on heart leaves are scattered, small grayish-brown necrotic spots (Fig. 2, C) or dense massing of small brown necrotic specklings, marginal wilt, and necrotic streaks on petioles, all of which advance to kill leaves. Other associated symptoms are malformation, distortion, crinkling, marginal curling of leaves, and bending of petioles and midribs (Fig. 2, D). Growth is stunted. Necrosis advances very rapidly to outer old leaves and to roots, and then plants are killed in 10 to 20 days.

**Eggplant.** Primary lesions are at first large, diffused chlorotic spots ( $\frac{1}{2}$  to  $\frac{3}{8}$  inch in diameter) with a small, faint brownish center (Fig. 3, A), and

<sup>13</sup> See footnote 9.

TABLE 3.—Transmission of the yellow-spot virus, by *Thrips tabaci*, from *Emilia* to various plants

Name	Variety	Plants tested	No. of replica-tions	No. of test plants			Suscep-tibility to the virus	Feeding of <i>T. tabaci</i>	
				From <i>Emilia</i>	Infected	Tested		Adult	Larva
<i>The susceptible group</i>									
Spinach	Virofay	3	24	14	38	18	high	good	good
Broad bean	Unknown	1	12	9	20	11	low	“	“
Celery	Golden Self Blanching	4	27	1	27	20	high	“	“
Potato	Bliss Triumph	2	7	5	28	15	medium	“	“
Eggplant	New York Improved	3	31	5	3	2	high	“	“
Bell Pepper	Chinese Giant	2	13	8	23	12	“	“	none
Tobacco	Turkish	3	27	9	“	“	very	poor	poor <sup>b</sup>
Tobacco	White Burley <sup>d</sup>	2	32	27	10	5	“	poor	poor
<i>Nicotinia glutinosa</i>	c	2	36	26	12 <sup>e</sup>	2	“	very	none
<i>Datura stramonium</i>	Unknown	1	14	13	24	17	“	poor	poor
Pettunia	Witloaf	9	84	19	“	“	good	good	good
Chicory	Large Green Curled	2	14	9	41	28	medium	good	“
Endive	Grand Rapid	2	18	6	23	10	“	“	“
Lettuce					20	9	“	“	“
<i>The non-susceptible group</i>									
<i>Convolvulus nudiflora</i>	Unknown	1	16	0	40	0	“	poor	“
and <i>C. venghalensis</i>	Large White Ribbed	3	15	0	12	0	“	good	“
Beet	New Zealand	3	18	0	34	0	“	very	“
Swiss chard	Unknown	1	12	0	“	“	“	poor	“
Spinach	Unknown	1	8	0	6	0	“	good	“
Cabbage	Unknown	2	20	0	17	0	“	good	“
Carrot	Summer	1	8	0	11	0	“	“	“
	Chrysanthemum								

<sup>a</sup> No recovery tests were performed because larvae could not survive on the plants.<sup>b</sup> Adults and larvae survived only on very young plants.<sup>c</sup> Mechanical inoculation infected one each out of 6 *Emilia* and 6 *N. glutinosa*.<sup>d</sup> Strains of Kelley and Lockwood were supplied by J. E. McMurtrey, Jr., U.S.D.A.<sup>e</sup> Two strains from Sweden and Peru respectively were supplied by T. H. Goodspeed, University of California.

TABLE 4.—Summary of characteristic symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci* under greenhouse conditions

Plants	Primary lesions	Systemic symptoms		References on spotted wilt, with which the equations were compared
		On leaves	On stems and petioles	
Spinach		Necrotic spots (Fig. 2, C), marginal wilt, and distortion (Fig. 2, D)	Necrotic streaks	Lethal
Broad bean	Leaves: Zonate necrotic spots (Fig. 2, A) Stems and petioles: Necrotic streaks (Fig. 2, B)	Zonate necrotic spots and marginal necrotic blotching	Necrotic streaks	(42), (43)
Celery		Necrotic spots with chlorotic margin (Fig. 2, E)	Necrotic pockets (Fig. 2, E)	(19); (20), (39)
Potato		Zonate necrotic spots (Fig. 2, F), and bronze rings and specklings	Necrotic streaks	(28), (41), (42)
Eggplant	Leaves: Concentric necrotic rings and zonate necrotic spots with chlorotic margin (Fig. 3, A, B)	Several types of mosaic mottling (Fig. 3, D, E)	Slightly retarded	(42)
Bell pepper	Leaves: Zonate necrotic spots (Fig. 3, C)	Concentric necrotic rings, zonate necrotic spots, and necrotic vein-outlining (Fig. 3, I, J, K); mottling and distortion (Fig. 3, L)	Stunted, sometimes lethal	(5), (41) (42)
Tobacco	Leaves: Concentric necrotic rings, zonate necrotic spots, and necrotic vein-outlining (Fig. 3, F-H)	Zonate necrotic spots (Fig. 4, J), necrotic vein-handing, distortion, and yellowing (Fig. 4, K)	Lethal	(5), (7), (30), (42)
<i>Nicotiana glutinosa</i>	Stems and petioles: necrotic streaks	Zonate necrotic spots (Fig. 4, A), necrotic blotching (Fig. 4, B), concentric chlorotic rings, yellow vein-outlining, and distortion with yellow vein-handing and vein necrosis (Fig. 4, C, D)	Discoloration	(30), (41), (42)
<i>Datura stramonium</i>		Necrotic spots, necrotic blotching, distortion, and yellowing (Fig. 4, F-H)	Stunted	(7), (30), (42)
Petunia		Necrotic spots, necrotic blotching, distortion, and yellowing (Fig. 4, F-H)	Retarded	(21), (28), (48)
Chicory		Necrotic spots, necrotic blotching, distortion, and yellowing	Retarded	
*Endive				
*Petticoe				

<sup>a</sup> Concentric chlorotic rings on fruits.<sup>b</sup> Malformation and necrosis on flowers and flower buds.

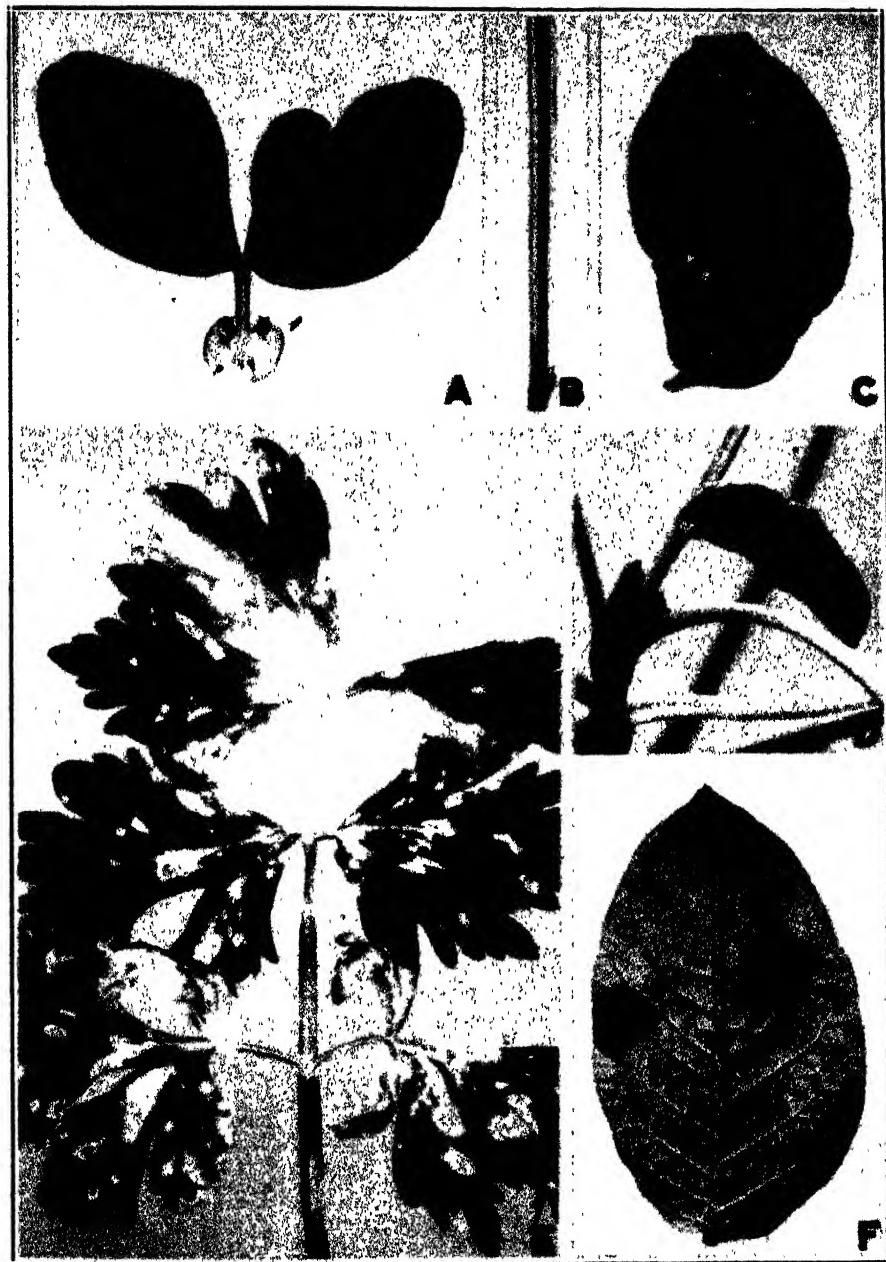


FIG. 2. Symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci*. A-B. Primary lesions on broad bean. Zonate necrotic spots on the laminae, and necrotic streak on the stem. C-D. Necrotic spots, marginal wilt, and abnormalities of the heart leaves of spinach (systemic). E. Necrotic spots with marginal chlorosis on the leaflets and necrotic pockets on the stem and petiole of celery (systemic). F. Necrotic spots with concentric zonation on potato (systemic).

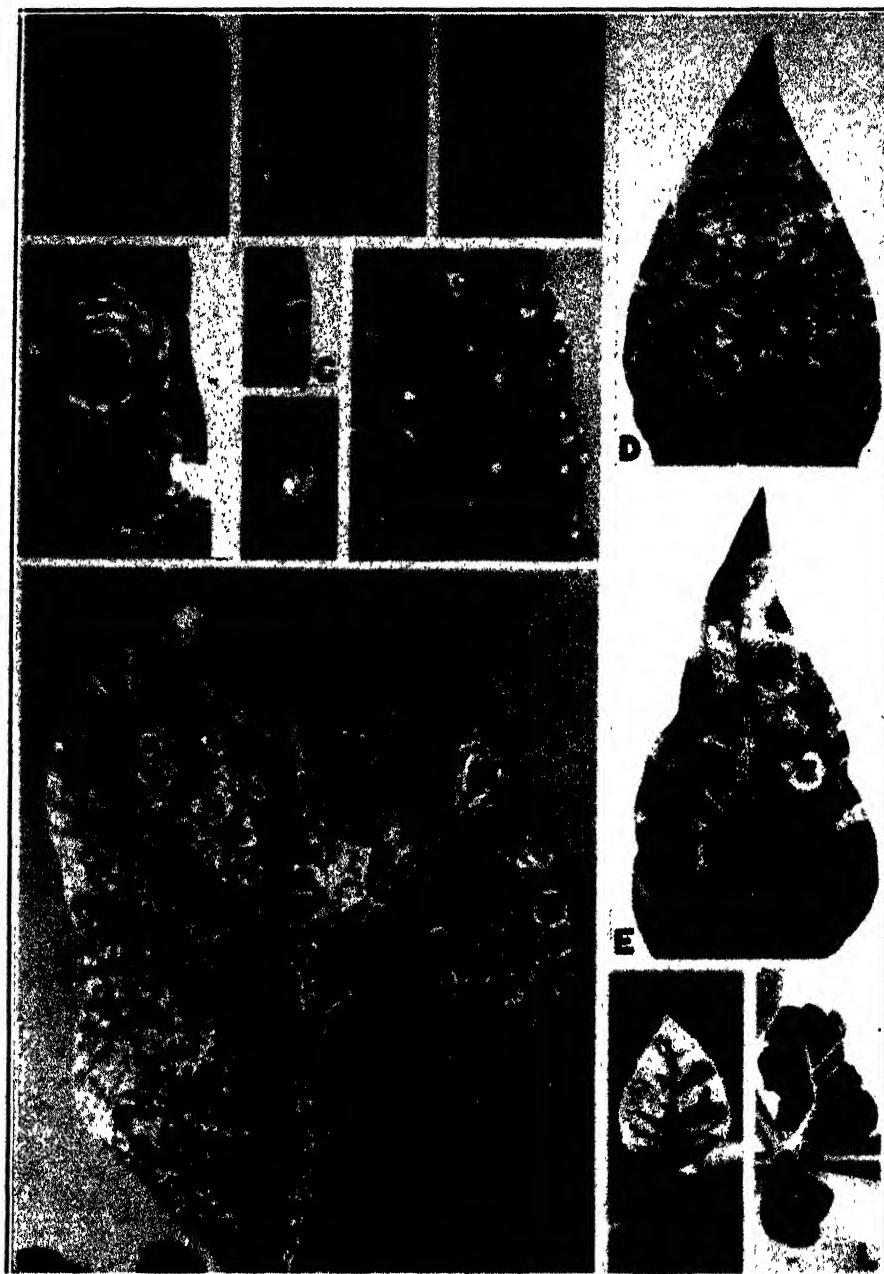


FIG. 3. Symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci*. A-B. Primary lesions on eggplant. Chlorotic spots advance to necrosis; B, enlarged. C-E. Bell pepper. C. Primary lesion. Enlarged zonate necrotic spot. D-E. Systemic symptoms: yellow mosaic mottling and coalescence of yellow and green concentric rings. F-L. Tobacco. F-H. Primary lesions: concentric necrotic rings and zonate necrotic spot with darker margin (F and H, enlarged). I-L. Systemic symptoms: I. Coalescence of zonate necrotic spots with darker margin. J. Coalescence of concentric necrotic rings and necrotic vein-outlining. K. Necrotic vein-outlining combined with vein necrosis. L. Abnormalities of the terminal leaves.

then a few broken, concentric bronze rings appear along outer margin which later rapidly advance to dark grayish-brown necrotic rings. Some necrotic rings advance to form solid spots with concentric zonation (Fig. 3, B), and others remain without further advancement after the chlorotic-spot stage. Leaves remain attached, and old necrotic spots sometimes show a broken, zig-zag-outlining necrotic ring. Growth is slightly retarded. Systemic symptoms were not detected.

*Bell Pepper.* Primary lesions are at first large, diffused chlorotic spots ( $\frac{1}{4}$  inch in diameter) and later advance to dark grayish-brown necrosis with concentric zonation, sometimes showing concentric outer bronze rings (Fig. 3, C). Systemic symptoms appearing on terminal young leaves, which are slightly distorted, are mosaic mottling with dense coalescence of small rings, spots, and concentric zonation, and show hardly any necrosis (Fig. 3, D). Later, very occasional leaves show, sporadically, other striking symptoms of coalesced, very large concentric chlorotic rings ( $\frac{1}{2}$  to 1 inch in diameter) (Fig. 3, E), or of single or coalesced, large, thick chlorotic rings ( $\frac{1}{2}$  to 2 inches in diameter) with diffused outer margin. Growth is slightly retarded. Sometimes fruits bear the symptoms of the concentric chlorotic ring type, resembling those on tomato fruits.

*Chicory and Endive.* Primary lesions were not observed. Systemic symptoms appearing on heart and young leaves are dark grayish-brown necrotic blotching at tips (die-back type), along margins and midribs, and in interveinal small areas (Fig. 4, F, G, H). Bending, curving, distortion, and yellowing are associated. On chicory, necrosis hardly expands and growth is only slightly hindered. On endive, however, necrosis expands rapidly under humid conditions and kills leaves; growth is severely retarded but plants are not killed.

*Nonsusceptible Plants.* *Commelina nudiflora* L. is infected by a mosaic in Hawaii, and this virus was experimentally transmitted by 3 species of aphids to pineapple (12), which is also susceptible to Y.S. virus. Attempts were made to demonstrate the possible relationships, if any, between both viruses and vectors. *C. nudiflora* and *C. venghalensis* L., which have not yet been tested for their susceptibilities to S.W. virus, were not infected by Y.S. virus. Another line of experiments (Table 5) indicated that *Commelina* mosaic was not transmitted by *Thrips tabaci* and that Y.S. virus was not transmitted by *Aphis gossypii* Glover, which is one of the vectors of *Commelina* mosaic, and that *Emilia* was not susceptible to *Commelina* mosaic.

TABLE 5.—*Tests of the transmission of Commelina-mosaic virus by Thrips tabaci and of yellow-spot virus by Aphis gossypii*

Viruses	Insects	Source plants	Test plants	No. of test plants	
				Tested	Infected
Commelina-mosaic	<i>T. tabaci</i>	<i>C. nudiflora</i>	<i>C. nudiflora</i>	37	0
	<i>A. gossypii</i>	<i>C. nudiflora</i>	<i>Emilia</i>	18	0
Yellow-spot	<i>A. gossypii</i>	<i>Emilia</i>	<i>Emilia</i>	24	0



FIG. 4. Symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci*. A-D. Systemic symptoms on *Datura stramonium*. A-B. Pale ring spots advance to necrotic blotching with coalescence of necrotic spots; A, enlarged. C. Abnormalities of the terminal leaves, appearing on the recently infected plant. D. The same, appearing on the plant infected for some time. E. Primary lesions on petunia. Zonate necrotic round spots with darker margin (enlarged). F. Systemic symptoms on chicory. Necrotic blotching and curving. G-H. Systemic symptoms on endive. Necrotic blotching. I-K. *Nicotiana glutinosa*. I. Primary lesion. Zonate necrotic round spot with darker margin (enlarged). J-K. Systemic symptoms: J. Coalescence of zonate necrotic spots. K. Abnormalities of the terminal leaves.

No infection of S.W. virus on beet, chard, and cabbage (20), and New Zealand spinach (*Tetragonia expansa* Murr.) (41) was reported. Similarly, these plants were not infected by Y.S. virus.

In spite of the fact that cauliflower is susceptible to S.W. virus (19, 44), Y.S. virus was not transmitted to 20 plants under the writer's conditions. Infection of S.W. virus on chrysanthemum was reported (1, 44, 45). However, a closely related vegetable, summer chrysanthemum (*Chrysanthemum coronarium* L.), was not infected by Y.S. virus.

*Field Infections.* Field-infected plants of potato, bell pepper, and chrysanthemum, bearing indistinguishable symptoms of yellow spot or spotted wilt, were observed. These are, in addition to the 9 species of plants besides pineapple, pea, and *Emilia*, found by Linford to be naturally infected<sup>14</sup> (35).

#### DISCUSSION

Former workers<sup>15</sup> (24, 25) listed 21 suspects to Y.S. virus. The present experiments demonstrated that 14 species of plants were infected and 8 escaped infection. Nine out of 14 suspects have not been previously recorded.

Among these 30 suspects, 18 are known also to be susceptible to S.W. virus; but the susceptibilities of the rest have not been tested, since they are mostly local weeds. The suspects to both viruses are the 14 species of plants here reported and pineapple, pea, *Nicotiana glauca* Graham, aster, and *Emilia sonchifolia*. The foregoing experiments demonstrated that the symptoms produced by Y.S. virus on these 14 species of plants, except spinach, endive, and chicory, for which no published descriptions are available for comparison, are identical with those of S.W. virus. A pineapple disease, presumably caused by S.W. virus in Queensland and South Africa, was reported to be indistinguishable from yellow spot (13, 23). The identity of the symptoms of the two viruses on *Emilia* and pea was established (24, 46, 49). Linford<sup>14</sup> stated that *N. glauca* and aster were infected by Y.S. virus and gave very brief descriptions of the symptoms. It is determined that his descriptions generally fit those of S.W. virus (5, 41, 42, 44).

Among the 8 species of plants that could not be infected, beet, chard, cabbage, and New Zealand spinach also are known to be nonsusceptible to S.W. virus (20, 41). The susceptibilities of two species of *Commelina* and summer chrysanthemum to S.W. virus are not known. In the entire present experiments, cauliflower was the only plant susceptible to S.W. virus and yet could not be infected by Y.S. virus. The writer, however, is convinced that the peculiarity of the conditions under which the experiment was performed was responsible for the failure of infection.

The general similarities of the host range and symptoms of the two viruses are thus well demonstrated, providing evidence for their coidentity.

The length of the latent period of a virus within a plant is specific for different viruses, and this character has been used by various workers to

<sup>14</sup> See footnote 8.

<sup>15</sup> See footnotes 8 and 9.

identify and classify a virus. The data on Y.S. virus obtained in the present experiments and also in the experiments of Linford (24, 25) are compared with those of S.W. virus compiled from the references (Table 6). Fair

TABLE 6.—Comparison of the lengths of the latent periods of infection of yellow spot and spotted wilt

Plants	Yellow-spot virus		Spotted-wilt virus		
	Primary lesions	Systemic symptoms	Primary lesions	Systemic symptoms	References
Spinach	Days	Days	Days	Days	
Broad bean	5-6	10-20	not available	not available	
Celery		10-12		not available	
Potato	8-10	30 <sup>a</sup>	14-16	25-28	(41, 42)
Eggplant	15		10		(42)
Bell pepper	15-20	30	21	not available	(41)
Tomato		8-27	7-8 <sup>b</sup>	7-21	(6, 15, 37, 42)
Tobacco	4-5	9-11	3-10	10-20	(5)
<i>Nicotiana glutinosa</i>	3-5	9-11	4-6	7-15	(5, 45)
<i>Datura stramonium</i>		10	2-3	8-10	(41)
Petunia	3-4	15-20		not available	(42)
Chicory		15-20		not available	
Endive		15-25	10	10-25	(2, 48)
Lettuce					
Pineapple	7-27 <sup>c</sup>		not available		
Pea	12-20 <sup>c</sup>		7-20		(49)
<i>Nicotiana glauca</i>		not available <sup>c</sup>	not available		
Aster		not available <sup>c</sup>	14-21		(42)
<i>Emilia</i>	8-27 <sup>c</sup>		not available		

<sup>a</sup> Datum from a single plant.

<sup>b</sup> The cases on very young plants were reported (6).

<sup>c</sup> Cited from Linford<sup>16</sup> (24, 25).

analogy on the majority of the plants is observed. This again is additional evidence for the coidentity of the two viruses.

The similarity of the mode of transmission of the two viruses has been demonstrated. S.W. virus is known to be transmitted by thrips vectors and mechanical inoculation. *Thrips tabaci* and 3 species of *Frankliniella* are vectors. *T. tabaci* transmits Y.S. virus but none of the above species of *Frankliniella* are distributed in Hawaii (35). Not only are both viruses transmitted by the same vector, but also the length of the incubation period within the vector and the specific stage of the vector for becoming infective are similar for both viruses (4, 5, 25, 30, 37, 42). The mechanical transmission of Y.S. virus, as easily accomplished as with S.W. virus, has been recently demonstrated by Parris (32). He also demonstrated that Y.S. virus is not seed-transmissible, similarly as in S.W. virus (10, 30, 37).

The similarities on the host range, symptoms, length of latent period within plants, and mode of transmission all suggest the coidentity of Y.S. virus with S.W. virus. The chemical and physical properties of S.W. virus

have been worked out with comparative thoroughness, but those of Y.S. virus are not known. However, since the technique of mechanical inoculation has now been established, these properties may be readily studied.

The outbreak of the tomato disease in Hawaii has been determined to be caused by the long-established Y.S. virus. Although this crop plant is most frequently and severely attacked by spotted wilt in all other areas, lower incidence of infection has been known in Hawaii. This may be attributed to the relative food preference of the insects, small acreage of tomato cultivation, and dissimilar distribution or centralization of the tomato cultivation and the viruliferous insect populations. However, this outbreak coincided with the disturbed host succession of the insects and planting of tomatoes within the infection center. The tomato field had been previously surrounded by potato patches that were harboring a high population of infected weeds and viruliferous insects. When these patches were plowed for the succeeding sugar-cane crop, the insects were compelled to disperse and concentrate in the nearby tomato field. There the insects were forced to feed on tomatoes, and, consequently, the outbreak suddenly appeared. A detailed study on migration of *Thrips tabaci* in relation to incidence of infection was published by Carter (14) and observation of a confirming incident also was reported by the writer (36).

#### SUMMARY

The yellow-spot virus was recovered by *Thrips tabaci* Lind. from field-infected tomatoes.

The yellow-spot virus was transmitted to and recovered from, by *T. tabaci*, spinach, broad bean, celery, potato, eggplant, bell pepper, tomato, tobacco, *Nicotiana glutinosa* L., *Datura stramonium* L., petunia, chicory, endive, and lettuce, which are known also to be susceptible to the spotted-wilt virus. The symptoms produced on these plants, except spinach, endive, and chicory, for which no published descriptions are available for comparison, were all identical with those of the spotted-wilt virus. The lengths of the latent period within the respective plants were also generally analogous. Beet, chard, cabbage, and New Zealand spinach, which are known to be not susceptible to the spotted-wilt virus, were also not infected by the yellow-spot virus.

These data provide clear evidence that yellow spot and spotted wilt are caused by the same virus.

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### MECHANICAL TRANSMISSION OF YELLOW-SPOT VIRUS: EVIDENCE FOR IDENTITY WITH SPOTTED-WILT VIRUS<sup>1</sup>

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(Accepted for publication Sept. 12, 1939)

In June, 1937, a diseased condition of tomato plants was observed at Waipahu, Oahu, Territory of Hawaii, characterized by necrosis of laminae and terminals, blotchiness of color and rugosity of surface of mature fruits, and a general cessation of growth (Figs. 1-3). Occasionally, slight bronzing of the upper surfaces of leaves was seen, prior to the appearance of necrotic lesions, and also bronzing of the stem ends of immature or partially mature fruits. A preliminary note recording the disease was published in 1938 (8).

The symptoms closely resembled those reported for spotted wilt on tomato with one exception, viz., bronzing was not outstanding. If one substitutes "necrosis" for "bronzing" in the descriptions of spotted wilt given by Samuel *et al.* (12), Bald and Samuel (1), and Smith (13), one has a fairly accurate and complete description of the disease as it appears in Hawaii, where it is common at present. Samuel *et al.* (12) have pointed out that bronzing is due to collapse and subsequent browning of the epidermal cells. Both of these terms signify necrosis. Furthermore, bronzing in Australia varies from an almost imperceptible glaze to so deep a bronze as to be almost black. It is faintest in glasshouse tomatoes or in very early field tomatoes, while in later field tomatoes *in the full summer sun*, discoloration is the darkest. These observations led Samuel *et al.* (12) to state, "This suggests some relationship between the development of bronzing and

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 50 of the Hawaii Agricultural Experiment Station.



FIG. 1. Effect of yellow spot on tomato in field. A and B. Terminal necrosis, stunting of plant, twisting and curling of leaflets and petioles. C. Growing point of diseased plant. Note intralaminar spot necrosis and twisting of laterals, petioles, and leaflets. Dark streaks present on stem do not show to advantage. Photograph C by K. Sakimura.

the intensity of light (or heat)." The tomato disease in Hawaii appears to be spotted wilt with bronzing largely suppressed by environmental conditions.

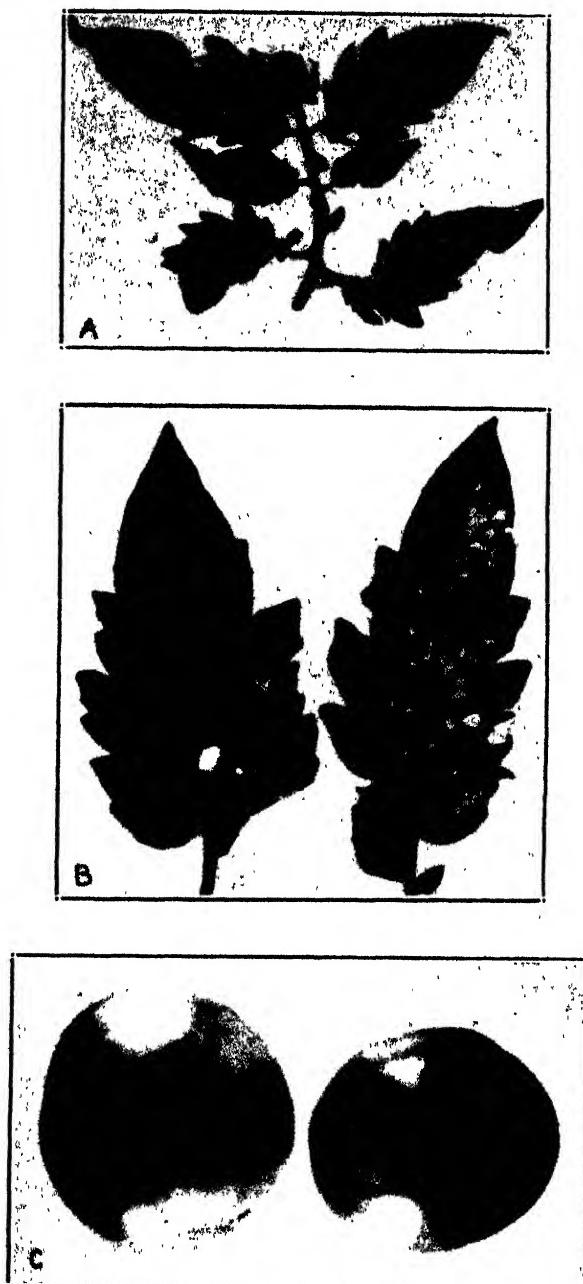


FIG. 2. Effect of yellow spot on tomato in field. A. Zonate banding pattern of necrosed tissue, accompanied by yellowing with little or no bronzing. B. More diffuse banding with increased yellowing. Severe necrosis at lower end of leaflet on right. C. Circular, yellowed areas, often zonately banded, in background of normal red surface of fruits. Photograph C by K. Sakimura.

M. B. Linford, plant pathologist of the Pineapple Experiment Station, Honolulu, T. H., first suggested to the writer that the local disease might be due to the virus of yellow spot of pineapple, for the symptoms closely resembled those obtained by Linford<sup>2</sup> on tomato (Fig. 4). The weed *Emilia sonchifolia* DC. (*sagittata*) had been reported by Linford (6) as a host of the yellow-spot virus, and examination of *Emilia*, growing in and around the tomato planting, where the disease was first observed, revealed that a large number of plants were diseased (Fig. 5).



FIG. 3. Mottling or "mosaic" effect of terminal leaves of plants which have been diseased for some time, or whose growth has been slow. Note two small necrotic lesions at apex of terminal leaflet.

#### REVIEW OF LITERATURE

The yellow-spot disease of pineapple was shown by Linford (6) to be caused by a virus, transmitted by *Thrips tabaci* Lindeman. According to this investigator, G. E. Paxton failed to transmit the disease from pineapple to pineapple by mechanical means. Suspects listed by Linford were pineapple, *Emilia sonchifolia*, and unspecified members of the families Bromeliaceae, Liliaceae, Caryophyllaceae, Leguminosae, Labiateae, Solanaceae, Rubiaceae, and Compositae. Sakimura (10) has since published Linford's list of host species. Carter (2) successfully transmitted the virus by mechanical means from pineapple to pineapple in a single instance.

That the viruses of yellow spot and spotted wilt are due to a single entity has been suggested by several investigators. Smith (13) has pointed out that symptoms on *Emilia* are very similar, and, according to Dr. Walter

<sup>2</sup> Unpublished data: cited by Sakimura (10).

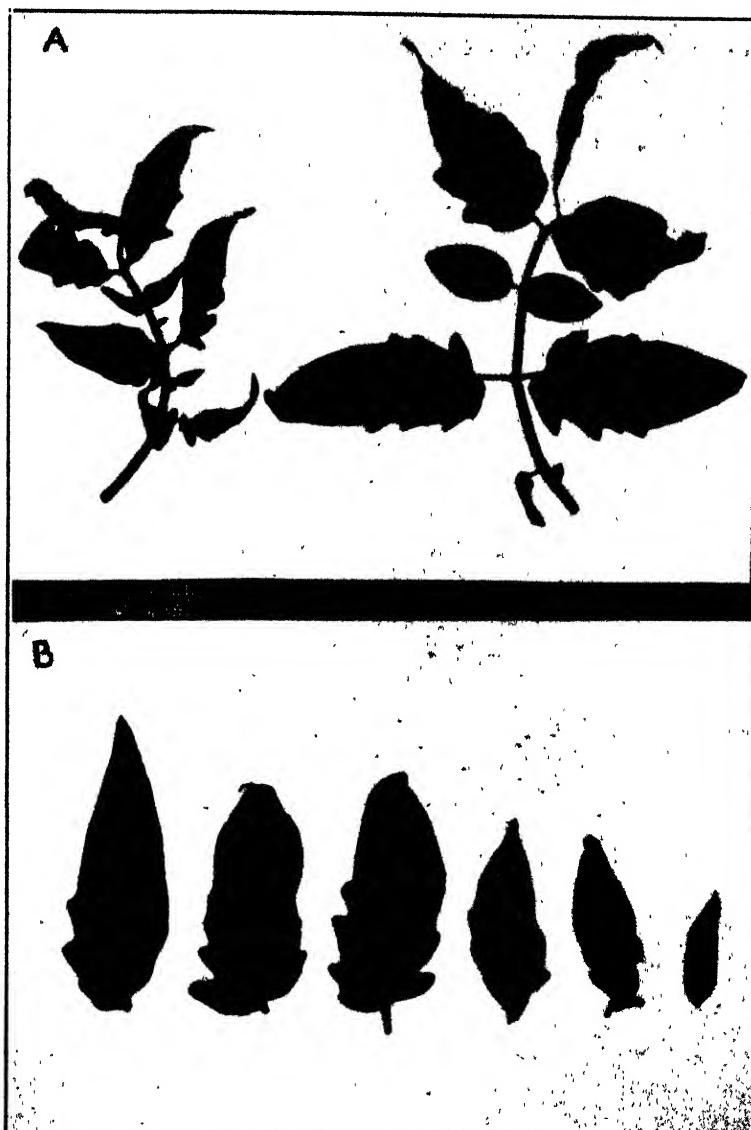


FIG. 4. Symptoms of yellow spot on tomato (var. Improved Stone) following inoculation, by *Thrips tabaci*, with the virus from *Emilia sonchifolia*. A. Distortion of leaflets and petioles and spot-necrosis and mottling of laminae. B. Necrosis of tips of leaflets and mottling of laminae. Healthy leaf at left. Photographs by M. B. Linford.

Carter,<sup>8</sup> entomologist of the Pineapple Experiment Station, Honolulu, T. H., symptoms of yellow spot on *Emilia* are indistinguishable from symptoms of spotted wilt on *Emilia* in California. Gardner *et al.* (4) recorded spotted wilt on *Emilia* in California but published no photographs. Linford (5) transferred the virus of yellow spot from *Emilia* by means of *Thrips tabaci*

<sup>8</sup> Oral communication.

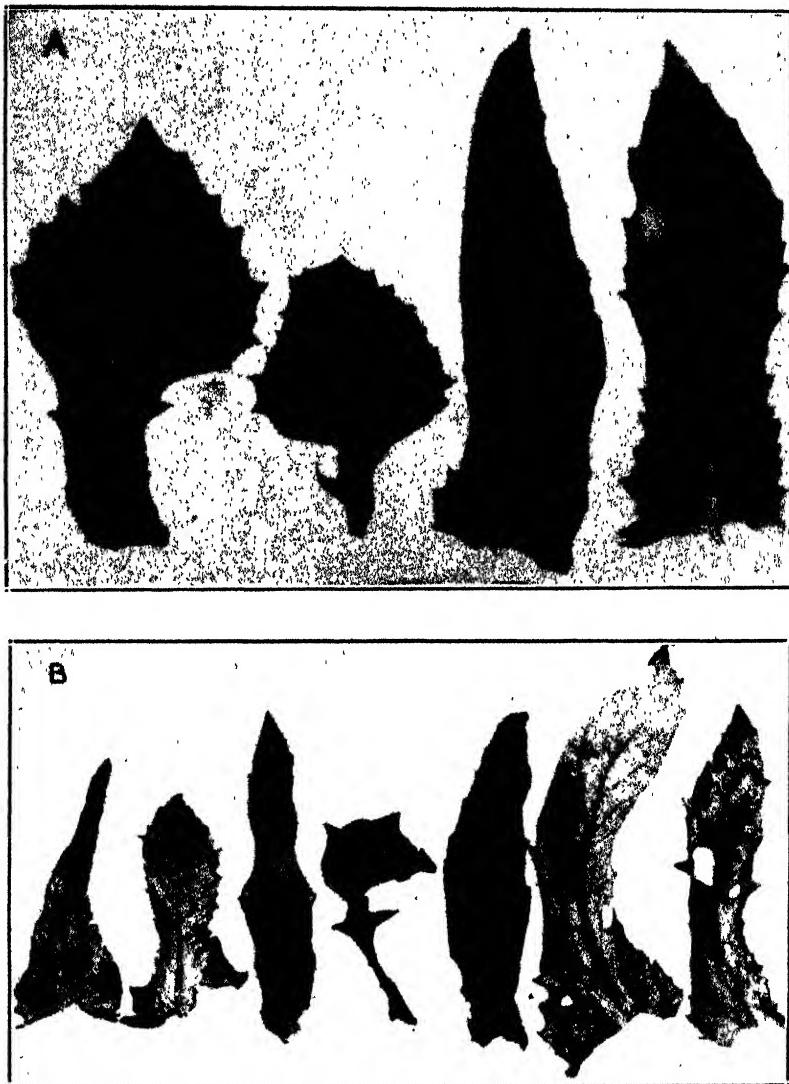


FIG. 5. Field symptoms of yellow spot on leaves of *Emilia sonchifolia*. A. Mottling and zonate banding with slight distortion. B. Pronounced chlorosis and distortion. Slight necrosis of third and fourth leaf from left.

to pea, not then recognized as a host of spotted wilt, to reproduce the symptoms of pea streak. He suggested that streak might be caused by the yellow-spot or a related virus. Later, Whipple (15) mechanically transmitted a virus from pea with streak symptoms, to tomato, *Emilia*, and other plants, and obtained symptoms identical with those caused by spotted wilt. Whipple pertinently states, "The demonstration that the tomato spotted-wilt virus causes a streak in pea is of peculiar interest inasmuch as the virus has much in common with that of the pineapple yellow spot in the

way of insect vector, incubation period, and the almost identical symptoms on *Emilia sagittata*." Snyder and Thomas (14) confirmed Whipple's work and conclusions.

The observations of Carter (3) on the association of yellow spot with *Kromnek* in the Union of South Africa, together with his citation of indications of identity of *Kromnek* and spotted wilt, obtained by Dr. E. S. Moore, further support the view that yellow spot and spotted wilt are caused by one virus.

This paper presents evidence that the local disease of tomato is caused by the same virus as yellow spot of pineapple.\*

#### EXPERIMENTS ON TRANSMISSION OF THE VIRUS

1. *Seed.* Seeds collected from immature and mature diseased fruits were planted in soil in the greenhouse and examined daily after emergence for symptoms of the local disease. Fifty plants were grown to maturity, and 650 plants until the emergence of the fourth compound leaf. In no case did disease symptoms appear.

2. *Mechanical Inoculation with Expressed Plant Sap.* Sap was extracted from the tissues of plant parts of diseased tomato and of other diseased plants, i.e., *Emilia sonchifolia* and potato, by grinding with sterilized mortar and pestle and filtering through cheesecloth. This preparation was then rubbed on the leaves of healthy plants of tomato, *E. sonchifolia*, potato (var. Bliss Triumph), bell pepper (*Capsicum annum*), garden pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), and lettuce (*Lactuca sativa*), with a glass rod. Carborundum powder was dusted on the leaves before abrasion as recommended by Rawlins and Tompkins (9). Three to 6 leaflets were inoculated when tomato was studied, and with other plants an equivalent area was treated. All plants inoculated were young and growing vigorously. For each series of inoculations an approximately equal number of plants was rubbed with juice from healthy plants and served as checks. Plants of both classes were grown side by side on the greenhouse bench, and none of the checks became infested.

The results of mechanical inoculations are given in table 1. The virus was transmitted mechanically from tomato to tomato and from tomato to potato, respectively, in 47 per cent and 75 per cent of the inoculations; from *Emilia* to *Emilia* and from *Emilia* to tomato, respectively, in 33 per cent and 24 per cent of the inoculations; and from diseased potato, following previous successful mechanical inoculation, to potato and tomato, respectively, in 57 per cent and 100 per cent of the inoculations. Negative results attended all attempts to transmit the virus by mechanical means from tomato to *Emilia*, lettuce, garden pea, or bell pepper. *Phaseolus vulgaris*, not a suspect of the yellow-spot virus (10) and an uncertain suspect of the spotted-wilt virus (13), also remained unaffected by mechanical inoculation.

\* Supporting evidence is being published concurrently (11).

TABLE 1.—Infections obtained in different species when mechanically inoculated with expressed juice from diseased *Lycopersicon esculentum*, *Cucurbita sonchifolia*, and *Solanum tuberosum*

Species of plant mechanically inoculated	Source of inoculum							
	<i>Lycopersicon esculentum</i>				<i>Emilia sonchifolia</i>			
	Latent period of infection		Latent period of infection		Latent period of infection		Latent period of infection	
	Plants inoculated	Plants infected	Time (days)	Number of plants	Plants inoculated	Plants infected	Time (days)	Number of plants
<i>Lycopersicon esculentum</i> var. Marglobe	93	44	1-10 11-20 21-30 31-40	8 9 13 14	25	6	11-20 21-30 31-40	2 1 1
<i>Emilia sonchifolia</i>	14	0			30	10	11-19	9
<i>Solanum tuberosum</i> var. Triumph	16	19	1-10 11-20 21-30 31-40	0 4 6 2				
<i>Lactuca sativa</i>	5	0						
<i>Pisum sativum</i>	11	0						
<i>Capsicum annuum</i>	6	0						
<i>Phaseolus vulgaris</i> var. La- miale	10	0						

Samuel *et al.* (12) report mechanical transmission of spotted-wilt virus from immature but not from mature diseased tomato fruit. In the present investigations, the local virus has been mechanically transmitted from immature tomato fruits in 5 out of 15 attempts. No transmission has been obtained with sap expressed from mature fruits with a similar number of inoculations.

The latent periods of infection of the virus in tomato, potato, and Emilia, respectively, are also shown in table 1. The younger and more vigorous the plant inoculated, the more rapid was the appearance of symptoms. This was particularly true of tomato.

Symptoms on tomato following mechanical inoculation (Fig. 6) corre-

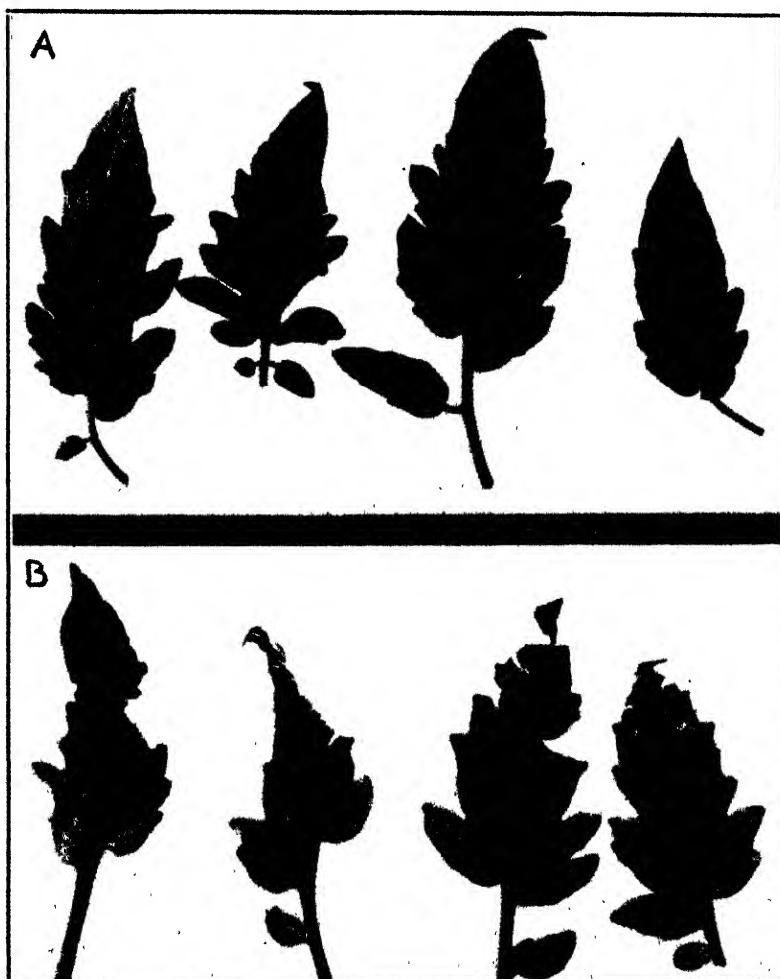


FIG. 6. Yellow spot on tomato leaves following mechanical inoculation. A. Necrotic lesions surrounded by yellow halo, with bronzing very slight or absent. B. Extreme necrosis and slight curling.

spond in intensity and type with conditions observed on plants naturally infected in the field and, in addition, are very similar to symptoms obtained by Linford with transmission by *Thrips tabaci*, previously mentioned and shown in figure 4. Necrosis of tips of tomato leaflets may accompany intralaminate lesions (Fig. 6, B). Fruits borne on diseased plants in greenhouse culture show the same type of blotchiness and rugosity of surface as exhibited in the field (Fig. 2, C). Mechanical inoculations are most successful with tomato when sap is extracted from the youngest tissues of recently diseased plants. It is very difficult to obtain positive results when sap is obtained from the old parts of diseased plants, or from any part of plants that have been diseased for some time or whose growth has been slow. The youngest parts of plants in this condition do not show typical symptoms: the foliage is mottled or "mosaicized," very much after the manner of typical tomato mosaic. Necrotic lesions are absent or of only minor significance (Fig. 3). Sap extracted from "mosaicized" leaves consistently gives negative results for the presence of a virus. This change in symptom expression and the failure to obtain a virus from "mosaicized" leaves are in accord with the findings of Samuel *et al.* (12) for spotted wilt. The transfer of "mosaicized" plants to fresh soil in larger containers causes the reappearance of the necrotic lesions on the leaves and petioles of the subsequently developed parts, and sap extracted from this new growth is highly infectious. Bald and Samuel (1) have noted that plants recently diseased are a much better source of virus than plants that have been diseased for a long time.

On the potato, systemic infection also occurs. One to 3 intralaminate, circular, concentrically zoned, necrotic spots appear on each leaflet; bronzing is slight or absent (Fig. 7, A). It is impossible to distinguish, without microscopic examination, between these spots and spots produced by the fungus *Alternaria solani* (E. and M.) Jones and Grout. Spores are usually present on fungous spots. In cases of severe infection the entire leaflet, or several leaflets and their petioles, may succumb (Fig. 7, B). Magee (7) has recorded the effect of the virus of spotted wilt of tomato on potato in Australia. Numerous circular, brown, dead areas are produced on the upper leaves of the plant, and longitudinal dead areas occur on the apices of the stems. The lesions are small and may show a characteristic type of zoning. Later, the spots on the leaves coalesce to form dead regions and the stems collapse at the apices. These symptoms closely approximate the effect of the virus of yellow spot of pineapple on potato.

On *Emilia*, the virus produces a distinct mottling or "mosaicized" effect of the young leaves, with subsequent development of circular, concentrically zoned spots on any portion of the laminae; these spots later may become necrotic (Fig. 8). Necrotic streaks may be produced on the petioles of diseased plants, and also on the peduncles and calyces of the inflorescences. Leaves may also be badly distorted and are often smaller than normal. These symptoms, with the exception of the necrotic spots, check fairly closely with

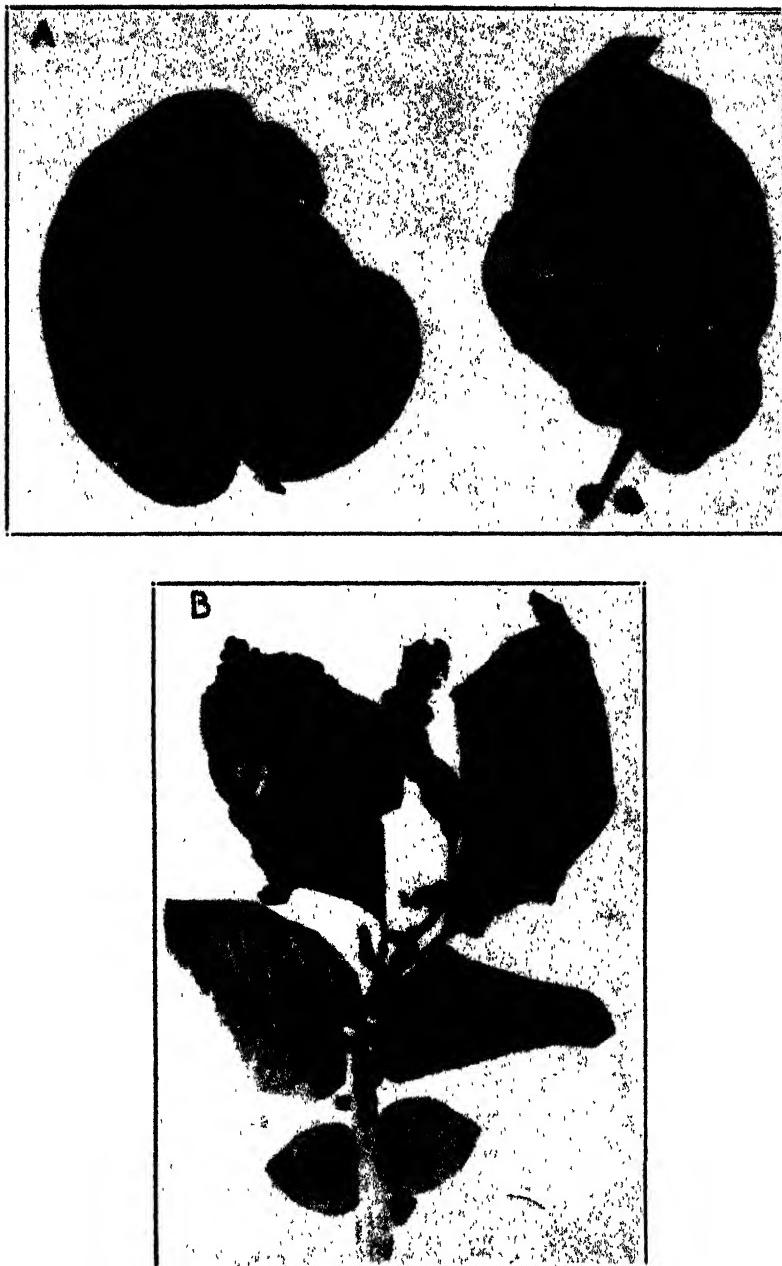


FIG. 7. Yellow spot on potato (var. Bliss Triumph) following mechanical inoculation. A. Necrotic lesions with zonate banding. B. Severe necrosis of several leaflets and portion of petiole. This type of reaction is seldom obtained.

the symptoms described on *Emilia* by Linford (6) for naturally diseased plants. This writer does not mention necrosis as a symptom of yellow spot on *Emilia*, but a careful search of diseased plants in the field has shown that

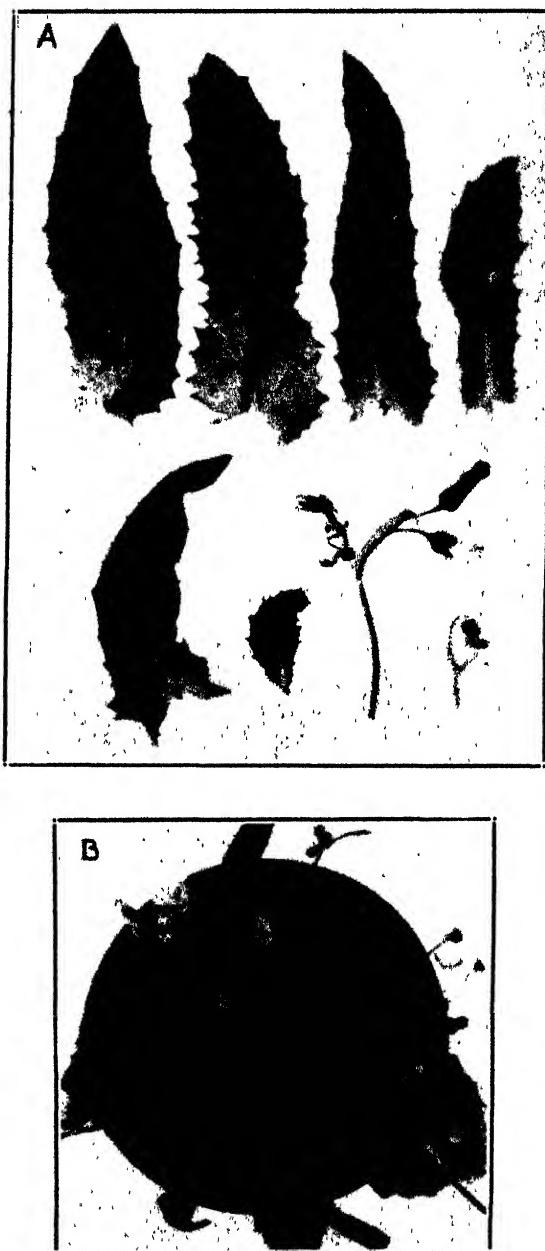


FIG. 8. Yellow spot on *Emilia sonchifolia* following mechanical inoculation. A. Zonately banded, chlorotic and necrotic spots on laminae, flower-pedicels, and calyces. B. Effect of virus on plant as a whole. Photographs by K. Sakimura.

occasionally necrosis may accompany the other, more typical, symptoms (Fig. 5, B).

3. *Grafting.* The virus has been successfully transmitted by grafting, as shown in table 2. Transmission occurred with all types of grafts tried,

TABLE 2.—*Results obtained by grafting scions of tomato and potato, diseased with the virus of yellow spot of pineapple, into healthy tomato and potato, and by grafting healthy tomato into diseased tomato*

Graft	Plants grafted	Plants infected	Latent period of infection	
			Time (days)	Number of plants
Naturally diseased tomato scion into healthy tomato	20	15	1-10	4
			11-20	3
			21-30	8
Mechanically inoculated diseased tomato scion into healthy tomato	15	12	1-10	3
			11-20	3
			21-30	6
Grafted diseased tomato scion into healthy tomato	2	2	11-20	2
Naturally diseased tomato scion into healthy potato	2	1	21-30	1
Mechanically inoculated diseased potato scion into healthy tomato	2	2	11-20	2
Healthy tomato scion into mechanically inoculated diseased tomato	1	1	11-20	1
Healthy tomato scion into grafted diseased tomato	3	3	11-20	3

infection ranging from 50 to 100 per cent. Symptoms on grafted plants are identical with symptoms observed on naturally infected plants in the field. The latent period of infection of the virus in grafted plants closely approximates that found with mechanically inoculated plants.

#### DISCUSSION

From the results obtained with mechanical inoculations and by grafting, it is concluded that the local disease of tomato is due to the virus of yellow spot of pineapple. Symptoms on tomato, *Emilia*, and potato are shown to be indistinguishable from symptoms reported for the effect of the spotted-wilt virus. Transmission studies by Sakimura (11) with *Thrips tabaci* show this to be true for other suspects. The spotted-wilt virus is transmitted mechanically and by grafting but is not seed transmitted. Data presented here show that the virus of yellow spot behaves similarly. These facts, together with the known relationship of pea streak to yellow spot (5) and to spotted wilt (14, 15), and of *Kromnek* to yellow spot and spotted wilt (3), points to a belief, expressed previously (13, 15), that the two viruses are identical, but physical properties have not yet been compared.

#### SUMMARY

1. A disease of tomato in Hawaii with symptoms identical with those of spotted wilt is described and evidence presented that the disease is due to the virus of yellow spot of pineapple.

2. The virus is not considered to be transmitted by seed, but is easily transmitted mechanically from tomato to tomato and potato, and from *Emilia sonchifolia (sagittata)* to *Emilia* and tomato. It has not been transmitted mechanically from tomato to *Emilia*, lettuce, garden pea, or bell pepper. The virus may also be transmitted by grafting, from tomato to tomato and potato and from potato to tomato. Symptoms artificially produced on tomato in greenhouse culture are identical with those found on naturally diseased field plants and are also similar to symptoms produced by Linford on tomato [unpublished : cited by Sakimura (10)] when the yellow-spot virus was introduced by the vector *Thrips tabaci*. Symptoms on potato are identical with those reported by Magee (7) for the virus of spotted wilt in Australia. On *Emilia*, the yellow-spot virus from tomato produces symptoms which closely resemble the effect of the virus on *Emilia* plants naturally infected in the field ; the symptoms are also very similar to those reported by Whipple (15) for the virus of spotted wilt on *Emilia* in Wisconsin.

3. The virus is easily recovered from immature, diseased tomato fruits, but not from mature, diseased fruits, a feature noted by Samuel *et al.* (12) for the virus of spotted wilt in Australia.

4. All available evidence points to the belief that the virus of yellow spot of pineapple is identical with the virus of spotted wilt, but physical properties have not yet been compared.

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# POTATO TUBER NET-NECROSIS AND STEM-END BROWNING STUDIES IN MAINE

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## INTRODUCTION

Net necrosis of the potato tuber (*Solanum tuberosum* L.) was described in 1914 by W. A. Orton with the name attributed to Wollenweber (5, p. 14). The fact that net necrosis is an occasional transitory symptom of leaf roll in certain varieties has been demonstrated by Schultz and Folsom (7), A. H. Gilbert (1, 2, and 3), and Quanjer and Elze (6). W. A. Orton described net necrosis as one form of internal stem-end browning, other forms of which were caused by fungi and bacteria. Since then various forms of internal discoloration with nonparasitic causes have been described in a literature that has become extensive and somewhat confused. In Maine and in the seed shipped from Maine, in addition to net necrosis (Fig. 1, A) occasional

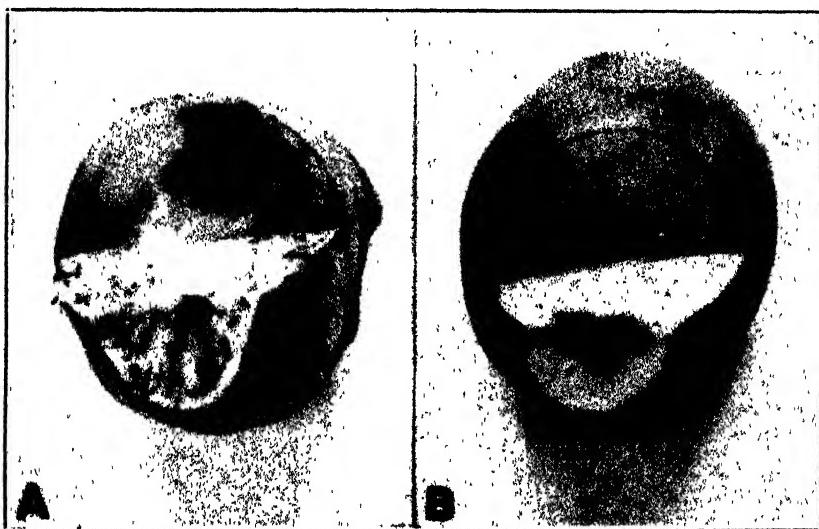


FIG. 1. Green Mountain potato tubers with part of the proximal (stem, butt, or navel) end cut off of each. The deep discoloration in tuber A is net necrosis, an occasional transitory symptom of leafroll in certain varieties, and the comparatively shallow discoloration in tuber B is another form of nonparasitic internal discoloration called "stem-end browning," in Maine.

outbreaks of another type of nonparasitic internal discoloration have given concern, chiefly because it apparently intergrades into net necrosis and because of the common dislike of cooks and seed buyers for any internal discoloration, even if of a type that is completely harmless. This second type of nonparasitic internal discoloration is called "stem-end browning," in Maine and in this paper. Stem-end browning (Fig. 1, B) was observed in

the winters of 1923-24 and 1929-30 and was common enough in some lots of potatoes during the winter of 1937-38 so that studies could be made of it both alone and in comparison with net necrosis. These studies included attempts to relate stem-end browning to different possible causes.

#### DIFFERENTIATION

Microscopically, stem-end browning usually can be distinguished from net necrosis through the former affecting both phloem and xylem and the latter affecting only the phloem. According to Hill (4) there are other differences.

Macroscopically, the two can often be distinguished with a high degree of success by an experienced person. Fairly successful attempts were made to develop an objective method useful to those with no experience. By recording the depth of penetration of the internal discoloration and its extent at a depth of about 12 mm., or  $\frac{1}{2}$  in., in the tubers, and observing the corresponding plants as grown later, it was found that discoloration in more than one ring or zone of the cross section of the tuber at a depth of 12 mm. usually was mild to severe net necrosis (that is, presaged leaf roll), while discoloration extending less than 12 mm. from the stem end of the tuber, or extending to that depth in only the xylem ring, usually was stem-end browning (that is, not associated with leaf roll). The results of one test are given in table 1, and illustrate the degree of accuracy with which the two diseases can be told apart by the half-inch-depth method. If the degree were perfect, the diagnosis of "probably net necrosis" would be accompanied by 100 per cent leaf roll instead of 87 per cent, and the diagnosis of "stem-end browning" would

TABLE 1.—*Accuracy of macroscopic differentiation of stem-end browning and net necrosis*

Diagnosis	Tubers	
	Total no.	Percentage leaf roll
Net necrosis	50	100
Probably net necrosis	143	87
Stem-end browning	286	23
Clear-fleshed	1206	14

be accompanied by 14 per cent leaf roll instead of 23 per cent. The degree is more nearly perfect when, to the rather arbitrary standard just described, there are added other factors on the basis of experience. Stem-end browning is usually darker colored than net necrosis. The discolored strands are fairly continuous in stem-end browning but discontinuous in net necrosis. Finally, net necrosis usually occurs in a greater number of concentric zones than does stem-end browning.

#### PREVALENCE AND IMPORTANCE OF THE DISEASES

According to the available records, net necrosis was rather scarce in Maine up to 1921 (7). Outbreaks of net necrosis and stem-end browning occurred in central Maine in the crop of 1923, and in central Maine and Aroostook

County in the crop of 1929. A limited survey during the winter of 1929-30 disclosed an average of 5 per cent net necrosis and 9 per cent stem-end browning in thirty stored stocks, with maxima of 16 and 44 per cent, respectively.

In the 1936 crop of potatoes, several stocks in Aroostook County were reported to have considerable stem-end browning. Of these, two had about 10 per cent of the tubers affected and two had about 20 per cent.

In the 1937 crop, an average of about 19 per cent of the tubers were affected with net necrosis in 40 bins of the Green Mountain variety in Aroostook County. Other varieties appeared less affected than Green Mountains. Katahdins and Chippewas have not yet been found with either net necrosis or stem-end browning. This varietal difference in susceptibility to these two types of internal discoloration is not correlated with relative susceptibility to leaf roll or to frost necrosis of the net type. Occasional small outbreaks have occurred in years other than those mentioned.

The infrequency of severe net-necrosis outbreaks in Maine is due to the infrequency of leaf-roll outbreaks in Aroostook County and to the fact that this County is the area of concentrated potato production in the State. In Maine, outbreaks of net necrosis occur more frequently outside of Aroostook County, in correlation with greater spread of leaf roll, and are as severe in percentage of tubers affected, but are less severe in aggregate loss because of the smaller total production of potatoes. Outside of Maine and certain other parts of New England the importance of net necrosis is greatly reduced, in the crop produced there, by the greater use of the less susceptible varieties and by the sale of the crop before the disease has had time to develop. However, wherever seed potatoes are shipped containing net necrosis, the disease is a warning of the presence of leaf roll and, therefore, is important.

#### RATIO OF NET NECROSIS TO LEAF ROLL

In one study, 29 Green Mountain stocks with 457 tubers apiece on the average were examined for internal discoloration in the tubers and for leaf roll in the vines produced from the tubers. There was a highly significant positive correlation ( $r = + 0.557$ ; see 9, table 16, on significant values) between the percentage of net necrosis (averaging 8.8) and the percentage of leaf roll (averaging 23.3) but the ratio between the leaf-roll and net-necrosis percentages in individual lots, averaging about 3.3 : 1, varied from 1 : 1 to 9 : 1. Therefore, while net necrosis in general may indicate how much leaf roll is present in a crop of Green Mountain potatoes, it is not a very reliable indicator of the percentage of leaf roll in all individual lots.

#### LACK OF EFFECT OF STEM-END BROWNING ON PLANT VIGOR AND YIELD

In the spring of 1937, 3 stored lots of potatoes on as many farms were drawn on for a comparison between plants from stem-end browning tubers and clear-fleshed tubers of the same stock.<sup>1</sup> Two-ounce seed pieces were taken

<sup>1</sup> Unless otherwise stated, the experiments described subsequently in this paper were conducted with potatoes of the Green Mountain variety and on Aroostook Farm, an experimental farm in Aroostook County.

from stem ends and bud ends of both kinds of tubers. Ten seed pieces of each kind were planted at 12-inch intervals in a 10-foot row, replicated in a total of 8 to 10 such plots. The results (Table 2) show that stem-end browning in the seed tuber had no effect on the yield rate of either stem-end or eye-end hills. Under the prevailing conditions, eye-end hills yielded at a significantly higher rate than stem-end hills. This difference between eye-end and stem-end hills was sometimes greater for the clear-fleshed tubers than for the internally discolored ones, which indicated further that stem-end browning is not injurious with respect to yield.

#### LACK OF PERPETUATION OF STEM-END BROWNING

Many tests over a number of years show neither more nor less stem-end browning in the crop grown from seed with the disease present, than in the crop grown in the same place from other seed free of the disease, with both crops stored in the same place. Also, the crop grown from seed with the disease present in all tubers, selected for the presence of the disease, usually has only a small percentage of affected tubers. Evidently the disease is neither perpetuated in a stock of potatoes, as are some parasitic and virus diseases, nor spread in the field with a consequent outbreak in storage, as occurs with leaf roll causing net necrosis.

On the other hand, stem-end browning appears to be more troublesome on certain farms than on others, and, on those farms, more abundant in certain

TABLE 2.—*Yield rate of hills grown from two-ounce seed pieces from stem ends and eye ends of clear-fleshed tubers and tubers with stem-end browning*

Seed-piece source, etc.	Yield rate in lbs. per hill, <sup>a</sup> etc.		
	Lot 1, Irish Cobbler	Lot 2, Green Mountain	Lot 3, Green Mountain
Stem-end browning tubers, stem end	1.47	1.47	1.70
Clear-fleshed tubers, stem end	1.50	1.50	1.67
Difference <sup>b</sup>	0.03	0.03	-0.03
Odds (to 1) <sup>c</sup>	1.00	1.00	1.00
Stem-end browning tubers, eye end	1.65	1.72	1.90
Clear-fleshed tubers, eye end	1.63	1.81	2.03
Difference <sup>b</sup>	-0.02	0.09	0.13
Odds (to 1) <sup>c</sup>	1.00	6.26	7.28
Stem-end browning tubers, stem end	1.47	1.47	1.70
Stem-end browning tubers, eye end	1.65	1.72	1.90
Difference in favor of eye end	0.18	0.25	0.20
Odds (to 1) <sup>c</sup>	Over 1350	Over 19,230	37.46
Clear-fleshed tubers, stem end	1.50	1.50	1.67
Clear-fleshed tubers, eye end	1.63	1.81	2.03
Difference in favor of eye end	0.13	0.31	0.36
Odds (to 1) <sup>c</sup>	1350	Very high	Very high

<sup>a</sup> At 1 lb. per hill and with spacing of hills 36 by 12 inches, the yield rate per acre would be 242 bushels or 88 barrels.

<sup>b</sup> Difference in favor of clear-fleshed tubers unless with -.

<sup>c</sup> If the odds are more than 30 to 1, the difference is considered to be significant.

fields than in others. Occasionally, there is a tendency to show progressively more net necrosis from one end of a bin to the other, but there is no such trend with stem-end browning in the same bins. It seems that some environmental condition or set of conditions in the field is the underlying cause of stem-end browning.

#### TESTING OF POSSIBLE CAUSES OF STEM-END BROWNING

During the outbreak of 1929-30 in Maine, several thousand tubers were examined in several dozen different lots. The stem-end browning was quite general and varied without any apparent relationship to soil, time of digging, presence of any virus disease, region of growth, place of storage, or origin of commercial strain, except that an early-dug lot and a spindle-tuber lot were included in the few lots that were free of the trouble.

In the course of the more recent work, out of the multitude of theories proposed as possible causes of stem-end browning, some of the more plausible were tested. The results will be described briefly.

#### Soil Type, Source, and Moisture

From 10 different farms where considerable stem-end browning had been found in the potatoes produced the year before, soil was taken that, when analyzed, was found to vary widely in regard to type, fertility, and organic matter content. The soil from each farm was used to fill 5 sections of chimney tile, each about 1 ft. square and 2 ft. long. Two potato plants were grown in each section of tile, fertilized at the rate of  $\frac{1}{2}$  ton of 8-16-16 fertilizer per acre. The tile sections were set upright in the ground near each other, on the experimental farm at Aroostook County. In the tubers, after a suitable period of storage, a very small amount of stem-end browning was found in nearly all lots, regardless of the soil in which they had been grown. The soil lots varied from 4.61 to 6.60 in pH, very low to excessive in  $\text{NO}_3^-$ , none to high in  $\text{NH}_4^+$ , very low to high in  $\text{P}_2\text{O}_5$ , very low to excessive in  $\text{K}_2\text{O}$  and Ca, low to very high in Mg, trace to high in Mn, trace to low in Fe, and very low to high in organic matter.<sup>2</sup> Apparently, these variations did not cause stem-end browning in the conditions of the test.

On the experimental farm in Aroostook County in the summer of 1937, which was a comparatively hot and dry season for the region, water was applied artificially to 2 rows of potato plants every other day. Samples of tubers from the watered rows and from neighboring dry rows both showed no stem-end browning in storage.

Three potato plants were grown in a loam of high humus content, in the greenhouse in 3 jars with the soil moisture kept nearly constant at 20, 30, and 45 per cent of the weight of the dry soil, respectively. In 2 other similar cultures the water content was caused to fluctuate between 20 and 45 per cent. All tubers produced by these 5 plants were free of stem-end browning.

<sup>2</sup> Analyzed by D. S. Fink, Associate Agronomist of the Maine Agricultural Experiment Station.

### Cumulative Fertilizer Treatment

"Permanent fertilizer plots" on Aroostook Farm had received fertilizer of different ratios from different sources or carriers, with and without lime, broadcast, with and without manure, etc., using different rotations, and had been receiving these same treatments for 10 years. The treatments were as follows:

<i>Rotation</i>	<i>Treatment</i>
Three-year	No fertilizer
Three-year	4- 8- 7—check
Two-year	4- 8- 7—check
One-year	4- 8- 7—check
Three-year	4- 8- 7—chemically pure salts
Two-year	4- 8- 7—broadcast
Two-year	4- 8- 7—plus 20 T. manure
Two-year	4- 8- 7—nitrogen in form of $(\text{NH}_4)_2\text{SO}_4$
Two-year	4- 8- 7—nitrogen in form of $\text{NaNO}_3$
Three-year	4- 8- 7—potash in form of $\text{KCl}$
Three-year	4- 8- 7—as preceding, plus 3000 lbs. ground limestone
Three-year	4- 8- 7—potash in form of $\text{K}_2\text{SO}_4$
Three-year	4- 8- 7—as preceding, plus 3000 lbs. ground limestone
Three-year	0- 8- 7
Three-year	2- 8- 7
Three-year	6- 8- 7
Three-year	4- 0- 7
Three-year	4- 4- 7
Three-year	4-12- 7
Three-year	4- 8- 0
Three-year	4- 8- 4
Three-year	4- 8-10
Three-year	4- 8-14

All tubers from these various plots in 1937 were free of stem-end browning.

Samples from many of these plots also had been examined several years previously, at which time no stem-end browning was found in more than two per cent of any sample.

### Deficiency in Sulphur, Boron, Iron, and Manganese

In 4 plots the addition of sulphur to the soil at different rates had brought the pH to 4.97, 5.40, 5.60, and 5.95, respectively. Samples from tubers grown here were similar with respect to stem-end browning, which was found to be present in a very low percentage of tubers.

On each of 2 farms that had produced affected potatoes in 1936, borax was applied to the soil in different rows at the rate of 1, 2.5, 5, and 10 pounds per acre, respectively, with each treatment replicated several times. There was no effect on the growth or yield rate of the potatoes, or on the amount of stem-end browning.

A nutritional experiment was started in a greenhouse in February, 1938. Plants were grown in white quartz sand in 2-gallon jars, using nutrient solutions. The nutrient solutions were made from chemically pure salts and were supplied by the constant-drip method (8). Plants were grown that became apparently deficient in boron, iron, manganese, and sulphur, respectively, corresponding to the types of solutions used. Other plants were grown that, apparently, had an excess of aluminum, arsenic, copper,

and chlorine, respectively. In addition to these, an iron-manganese relationship study was carried on. The plants received the following 9 treatments:

<i>Iron</i>	<i>Manganese</i>
Low	Low
Low	Medium
Low	High
Medium	Low
Medium	Medium
Medium	High
High	Low
High	Medium
High	High

All the plants grew fairly well in the sand with the exception of those deficient in boron and those injured by an excess of arsenic or copper; these three sets were considerably stunted. After about 100 days from planting, no more moisture was supplied, so that the plants wilted. The tubers were examined for stem-end browning about a month later, but none of them showed it.

In another experiment several 2-gallon jars were filled with soil from different farms that had produced potatoes with stem-end browning. Others were filled with soil known to be deficient in boron for the production of rutabagas. Chemically pure salts were added to each jar to supply nitrogen, phosphorus, and potash at the rate of 3000 pounds of 4-8-8 fertilizer per acre. One potato plant was grown in each jar. Water was added as needed. No stem-end browning developed in the tubers.

#### Injury to Plants

The opinion has been expressed that stem-end browning is caused by some form of mechanical injury to the plants; so several methods were used to injure either the tops or the roots. One method employed was late cultivation. After the plants had grown too large to be cultivated without disturbing the roots considerably, some of them were cultivated with as little injury as possible; others were cultivated as close to the plants as possible, breaking off a great many of the roots; and others were left uncultivated as a check. Another simple but harsh method was to jerk each plant upward until some of the roots were broken giving a cracking sound. About half of the foliage was removed from other plants during the latter part of the growing season. Another method of injury tried was to hoe as much dirt away from the plants as possible, leaving the roots and tubers protected from the sun and heat by only a very shallow layer of dirt. Samples were dug and placed in storage from plants killed by late blight. Another block of plants was sprayed late in the season with a sulphuric-acid solution sufficiently strong to kill them. When the tops were thoroughly dead the potatoes were harvested and stored. Other samples were dug and placed in storage from plants that were killed or severely injured by frost. Samples

were dug and stored also from plants that were still green and appeared to be normal in every way.

Except for late cultivation, frost injury, and digging when green, the treatments were all carried out in the same field, and a small amount of stem-end browning was found in each case regardless of treatment, but no more than was found in the untreated check lots. No stem-end browning was found in any of the samples from the field in which the tests were carried out on frost injury and digging when green. Late cultivation had no effect on the development of stem-end browning.

Potted plants were frozen. The tubers when examined several weeks later showed no stem-end browning.

#### Time and Manner of Digging

Twenty-eight hills of potatoes were dug by hand and each was stored separately. Only 1 or 2 tubers from any hill showed stem-end browning when they were examined in the winter. About half the hills were thus affected. However, the percentage of tubers affected was no higher than where the potatoes were dug by machine and stored in bulk.

#### EFFECT OF STORAGE DURATION AND TUBER WEIGHT

During the winter of 1937-38, one lot of potatoes, of which about 20 per cent had stem-end browning and about 5 per cent had net necrosis, was divided into several similar parts. The tubers were washed and weighed to the ounce and the tubers of each weight-class were divided equally among the several parts, so that the distribution of tuber weights was identical for the several parts.

A second lot of tubers, with about 9 per cent stem-end browning and about 22 per cent net necrosis, was divided in the same way.

At successive dates during the winter, one of the identical parts of each lot was used to determine whether or not there had been any increase in the amount of stem-end browning or net necrosis. Each disease was recorded as to percentage of tubers affected. In addition, a more objective record of discoloration was made by determining the depth of discoloration both in terms of absolute thickness of the affected part of the stem end and in terms of percentage of total tuber length included in the thickness of the affected part, and by estimating the extent of discoloration in the cross section evident at a depth of about 12 mm. from the stem end. For example, a tuber might have a record of discoloration extending 20 mm. or 16 per cent of the length of the tuber and involving, at the 12-mm. depth, 0.3 of the vascular ring and 0.4 of a radius of the cross section.

By no criterion was there any increase in internal discoloration after the time the study was begun, about December 15. However, the results are only preliminary, because the storage room was not kept at a constant temperature, the successive samples contained only about 250 and 350 tubers each, the study was not started early enough in the season, and seasons may vary.

With each weight-class of tubers divided equally among the several parts and, therefore, treated like every other weight-class during the storage period, the records on all tubers were recombined to determine the relationship between tuber weight and internal discoloration. The results are given in tables 3 and 4 and show that stem-end browning increased in frequency as tuber weight decreased, while net necrosis, where abundant, increased in frequency as tuber weight increased.

TABLE 3.—*Frequency of internal discoloration in weight classes of tubers*

Lot	Item	Weight class and item expressed numerically				
		1 to 3 oz.	4 oz.	5 oz.	6 oz.	7 and more oz.
1	Total tubers	512	562	327	244	388
	Percentage of tubers with stem-end browning	24.8	24.4	18.7	20.1	14.9
	Percentage of tubers with net necrosis	3.6	6.0	3.9	3.7	5.4
2	Total tubers	974	357	237	172	351
	Percentage of tubers with stem-end browning	11.2	9.2	6.3	5.2	4.8
	Percentage of tubers with net necrosis	12.0	21.0	31.2	27.7	39.3

TABLE 4.—*Average tuber weight of tubers with different types of internal discoloration*

Kind of tubers	Average weight of tubers in ounces	
	Lot 1	Lot 2
With stem-end browning	4.61	3.56
All kinds	4.95	4.27
With no discoloration	5.03	3.97
With net necrosis	5.03	5.56

#### SUMMARY

Net necrosis of the potato tuber, an occasional transitory symptom of leaf roll in certain varieties, is distinguishable in several ways from another kind of nonparasitic internal discoloration called, in Maine, "stem-end browning."

While an outbreak of net necrosis indicates unusual spread of leaf roll in the preceding growing season, the ratio of net necrosis to leaf roll varies greatly with individual seed stocks.

Stem-end browning occurs more in some seasons than in others in any part of Maine, and, when occurring, is usually more abundant in some parts of Maine than in others. It has no effect upon plant vigor and yield rate and is not perpetuated in the tubers from one generation to the next. Through successive seasons it appears more troublesome on certain farms than on others, and in certain fields of those farms than in others.

No correlation was found between stem-end browning and various possible causes such as soil type, previous occurrence in the soil, previous fertilizer treatment of the soil, soil nutrients, pH of the soil, soil water, presence of virus disease, origin of commercial strain, injury to the parent plants, time and manner of digging, and certain storage conditions.

Within a given stored lot, stem-end browning was correlated negatively with tuber weight, and net necrosis was correlated positively. Neither malady increased after December 15 during the winter of 1937-38.

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### A TRANSMISSIBLE LEAF-CASTING YELLOWS OF PEACH

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The disease here considered first attracted our attention in Green Valley, Solano County, California, in 1932. It has since been seen in several orchards in that valley and in the neighboring Suisun Valley, as well as in 2 orchards in Contra Costa County. Similar symptoms have been seen in 4 other counties. The disease has been seen on Early Crawford, Elberta, Fay Elberta, Muir, and Orange Cling.

There is evidence of appreciable spread in some orchards. In a block of 117 trees, where 37 trees were found affected in August, 1936, symptoms had been seen on a total of 49 trees up to mid July, 1939.

#### SYMPTOMS

The only tangible early-season symptom of this disease recognized thus far is a slight delay in the time of blossoming of affected trees.

In the Green Valley area, where most of the observations have been made, growth may appear approximately normal, especially in the lower part of the tree, until toward the end of June. The distal parts of severely affected trees or branches usually make little or no growth but fail to present more

specific symptoms during the spring months. Eventually such branches die back, often accompanied by sunburn and invasion by shot-hole borers. A single tree may bear some severely affected branches and others, which are normal in appearance, even after the disease has been present in the tree for several years.

More specific symptoms are seen in leaves from late June through July and August. Irregular portions of the leaf blade turn pale-green to yellow and become brittle, soon separating from the rest of the leaf (Fig. 1). Such areas often are surrounded by a purplish border. Affected leaves gradually take on a greenish yellow cast throughout, become somewhat rolled upward and, beginning with the oldest leaves, are dropped until the entire shoot is bare (Fig. 2). Defoliation may be complete on individual branches by the end of July. Tufts of new leaves may appear later at the tips of shoots in some situations.



FIG. 1. Leaf symptoms on naturally affected peach, collected in the field, June 20, 1939.

A swelling of the larger veins is commonly present on affected plants in the greenhouse but is rarely seen in the orchards.

More or less coincident with the dropping of leaves, the fruit on affected branches shrivels and drops; on a single tree as harvest approaches there may be on the ground or on the tree all sizes of fruits from small shrivelled mummies to those normal in appearance.

#### RELATION TO DISEASES OF PEACH IN OTHER AREAS

The characteristics of the disease in California agree closely with those



FIG. 2. Characteristic appearance of affected shoots from middle to latter part of summer. Many shoots are entirely defoliated at date of this collection, September 8, 1937.

described by Blodgett<sup>1</sup> in Idaho. He is of the same opinion after having seen some of the affected orchards in California. There is close agreement also with the "X" disease of peach described by Stoddard<sup>2</sup> in the North-

<sup>1</sup> Blodgett, E. C. Fruit diseases in Idaho, 1936. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 21: 89-95. 1937.

<sup>2</sup> Stoddard, E. M. The "X" disease of peach. Connecticut (New Haven) Agr. Exp. Stat. Circ. 122. 1938.

eastern States, with two exceptions, namely that all the leaves of affected shoots are dropped in the California orchards including those at the tips and that the percentage of infections when inoculations are made from peach to peach is low with the disease in California. It is probable, however, that the diseases are the same or very closely related.

#### APPARENT RELATION TO CHERRY BUCKSKIN

The leaf-casting disease of peaches was first noticed in a block of peach trees adjacent to a cherry orchard severely affected by the buckskin disease, the symptoms of which have been described.<sup>3, 4</sup> More recently cases have been found of association between affected cherries and peaches in 4 separate districts in 2 counties. Partly for this reason, 7 peach trees were inoculated early in 1932 with cherry scions affected by buckskin. When no symptoms appeared in 1932, the same trees were reinoculated in 1933. The inoculated trees continued healthy in appearance in 1934 and 1935, but, in 1936, 4 of them developed symptoms typical of the natural infection on peach, while 3, kept as controls, remained healthy.

This result prompted 2 other experiments, which were made with trees planted early in 1937 and 1938, in each of which 10 Fay Alberta trees were inoculated with buckskin-cherry scions and 10 trees of each lot were left as controls. Here again none of the inoculated trees developed symptoms during the first growing season after inoculation, but, up to October, 1939, 3 trees in one inoculated lot and 5 trees in the other developed clear symptoms of the peach disease, while the 20 control trees remained healthy.

In 1932, 10 cherry trees were inoculated with scions from diseased peach trees. The same 10 trees were again inoculated in 1933 by the same means. None of these inoculated cherry trees showed symptoms of buckskin until 1939 when 2 of the trees showed distinct symptoms of the disease. Since control cherry trees frequently became infected, these results are not considered significant.

In several experiments in the greenhouse a total of 27 apparently healthy cherry trees (*Prunus avium*), including 9 of the Napoleon variety, have been inoculated by inarching or grafting with scions of naturally affected peach. Altogether, 8 of these cherry trees have died, some of them rather suddenly and others after failing to make any growth for several months, but, since the more characteristic symptoms of buckskin are not discernible under the conditions of these tests, the death of the plants is not attributed with certainty to the inoculation. We are, therefore, still uncertain as to the transmissibility of the disease from peach to cherry.

#### INOCULATION OF OTHER SPECIES OF PRUNUS

Twenty-five plants of the western chokecherry, *Prunus demissa*, transplanted from the wild to pots, were inoculated from peach. Some of the

<sup>3</sup> Rawlins, T. E., and W. T. Horne. "Buckskin," a destructive graft-infectious disease of the cherry. *Phytopath.* 21: 331-335. 1931.

<sup>4</sup> Rawlins, T. E., and K. G. Parker. Influence of root-stock on susceptibility of sweet cherry to buckskin disease. *Phytopath.* 24: 1029-1031. 1934.

grafts were made in November, 1938, and others in February, 1939. The survivors of this experiment grew poorly and all failed to show any clear symptoms up to mid-October, 1939, though one of them bore doubtful symptoms at this time.

During February, 1939, 7 potted chokecherry trees were inoculated with buckskin sweet-cherry scions and 6 were grafted with healthy sweet-cherry scions. Seven were left ungrafted. Of the 7 inoculated trees 2 died and 4 showed a conspicuous carmine (Ridgway) color on the lower leaves when examined on October 2, 1939. This color was more or less continuous along the edges of the leaves and between the main lateral veins. The upper side of the mid-rib and the main lateral veins remained green. None of the 13 control trees showed this symptom distinctly; some did show a tinge of this color in some of the lower leaves. There is some evidence that the reddening of the diseased leaves is preceded by a chlorosis. Since the X disease virus of peach in the Eastern States is reported to cause a reddening of the leaves of infected chokecherries,<sup>5</sup> our results may perhaps be considered as further evidence that the buckskin disease is identical with or closely related to the "X" disease.

*Prunus demissa*, regarded by some botanists as a variety of the eastern chokecherry, *P. virginiana*, has not been found thus far in California in the vicinity of affected peach or cherry orchards.

The only native *Prunus* species known to occur in such association are *P. ilicifolia* and *P. subcordata*. The latter has not been inoculated with the peach disease in sufficient numbers even to be suggestive. Both have been inoculated on a small scale with cherry buckskin, but with negative results.

Ten or more plants of each of the following species were inoculated by grafting with affected peach scions, most of them in September, 1938: *Prunus armeniaca*, *P. cerasifera*, *P. communis*, *P. ilicifolia*, *P. mahaleb*. The results to date are negative, but, in view of the low incidence of infection in all the inoculation experiments (see below), cannot be regarded as conclusive.

A few almond and plum trees and a larger number of apricot trees have been seen in close proximity to affected peach trees in orchards, but without any symptoms to suggest that these are affected by the peach disease.

#### INOCULATION EXPERIMENTS WITH PEACHES

From the beginning, the number of infections resulting from inoculation by grafting peach to peach was low. It was at first assumed that the virus had not had sufficient time to pervade the tree from which the inoculum was taken. When small branches, marked in autumn as bearing symptoms, were found to be uncertain sources of virus the following winter, it became apparent that some other explanation was required. Even the scions used as inoculum often failed to develop symptoms, as well as to infect the inoculated trees.

<sup>5</sup> See footnote 2.

The results of inoculations and the erratic distribution of symptoms through trees known to have been infected for several years suggested that the virus is not completely systemic. This view is supported by the results of an experiment in which scions were taken from 3 small branches that arose from the same side of a larger branch within a distance of 22 inches. The middle one of these branches bore symptoms in September, 1937, while the other two did not. In February, 1938, 4 scions from the lower branch and 10 from each of the other two were grafted on potted peach seedlings. Up to September, 1939, 3 plants inoculated from the visibly affected branch had developed symptoms, while only 1 plant in the two other groups appeared to be diseased.

Of a few affected shoots or small branches marked in the autumn of 1937 and left on the tree, at least 1 or 2 failed to develop symptoms in 1938. Following this observation, 70 shoots exhibiting clear symptoms were marked in September, 1938, on trees of Early Crawford, Fay Elberta, and Orange Cling. Thirty-three of these were found on July 24 and August 15, 1939, and 11 of these, including some of each variety, appeared to be entirely free of symptoms. This suggests that the virus does not necessarily persist, even in branches that have been invaded by it. Only one case of apparent recovery of an entire tree has been seen, however, and this is still in some doubt.

Several tests were made with roots as a source of the virus. In one of these, 3 pieces of small roots from a diseased orchard tree were grafted on each of 5 nursery trees, which were then planted in a field plot. Five similar trees were inoculated at the same time by grafting affected scions on the tops. After 2½ years 2 of the trees inoculated from shoot scions had shown symptoms, while none of those inoculated by roots had done so, although some of the root pieces used as inoculum were alive for at least 1 year after inoculation.

In another test, pieces of roots from diseased trees were grafted on the tops of 10 seedling peach trees in the greenhouse, and ten similar trees were inoculated by scions from shoots. At the end of 12 months, 4 trees inoculated from roots and 6 inoculated from shoots had developed symptoms. Thus far the roots seem to be less satisfactory than shoots as inoculum.

A few tests have indicated that the Orange Cling variety is a better source of virus than several other varieties. For example, 10 seedling peach trees were inoculated at the same time in the greenhouse with scions of each of the varieties Early Crawford, Fay Elberta, and Orange Cling. During 9 months from the time of inoculation, 3 trees inoculated from Orange Cling displayed symptoms, while none were seen on trees of the other 2 lots.

An experiment was made to test the effect of susceptible and resistant stocks on the expression of symptoms. Successive scions from affected shoots were grafted alternately on potted plants of peach and myrobalan (*P. cerasifera*), i.e., the scion from the base of a shoot was grafted on peach, the next on myrobalan, the third on peach, etc. All told, 50 scions were grafted in

this way. Sixty-five days later, 9 of the scions on myrobalan had developed initial symptoms as against 2 of those on peach roots. Soon afterwards, however, this difference became less striking.

#### SUMMARY

A graft-transmissible leaf-casting yellows disease of peach, *Prunus persica*, is established in several counties in central California. It is similar to if not identical with diseases of peach in other areas, including the "X" disease of the Northeastern States. There is evidence also that the disease may be caused by the same virus as the buckskin disease of sweet cherry, *Prunus avium*.

Observation of marked branches and grafting experiments indicate that the virus usually is not completely systemic in the tree and is not always present in branches known to have been affected in earlier years. Roots seem to be somewhat less effective than tops as sources of the virus.

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### A SOFT ROT OF APPLES CAUSED BY TRICHOSEPTORIA FRUCTIGENA

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While collecting diseased apple fruits for class study, H. H. Whetzel of Cornell University, selected among others 2 severely infected McIntosh apples. The fruits had well-developed soft-rot lesions, partially covered with the black, slightly erumpent pycnidia of a fungus (Fig. 1). The color of the skin over the lesions was vinaceous-fawn,<sup>1</sup> glossy and sunken. The infected flesh of the apples was brown and similar in consistency to apples invaded by *Penicillium expansum* Link. A distinct margin separated the diseased and healthy tissues.

As the symptoms conformed to none of those of the common apple fruit rots, the specimens were given to the writer for further study.

#### THE CAUSAL ORGANISM OBTAINED IN CULTURE

A fungus was obtained in pure culture by planting small pieces of the infected apple tissue on potato-dextrose agar (1.0 per cent) and by pouring dilution plates. The fungi obtained by the 2 methods were identical, and it was later demonstrated that the true causal organism had been isolated. The fungus grew and fruited well on the agar medium, but its pycnidia (Fig. 2, A) were distinctly different in color and shape from those found on the original apple fruits (Fig. 2, B).

<sup>1</sup> Ridgway, Robert. Color standards and color nomenclature. 43 pp., illus. (Washington.) 1912.

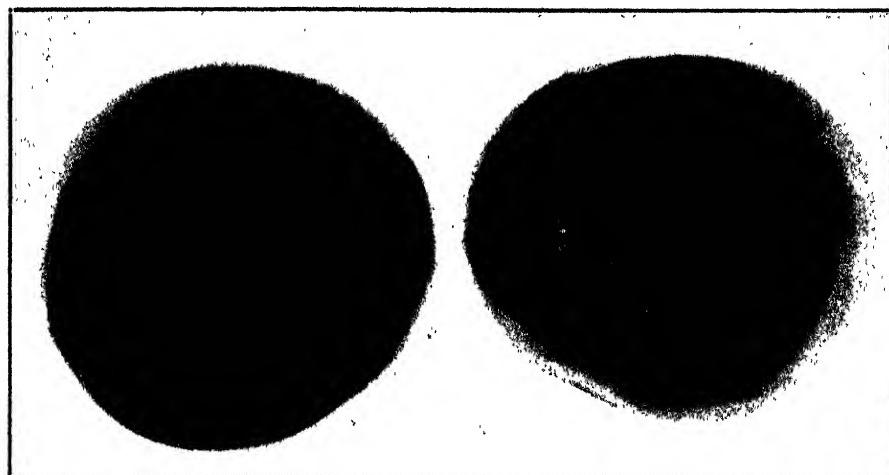


FIG. 1. McIntosh apples on which the pathogen was originally found.

When microtome sections ( $18 \mu$ ) of the pycnidia were cut and examined, it was found that the erumpent forms taken from the agar culture were covered with a mass of greyish hairs. The pycnidia taken from the apple fruits were nonerumpent or only partly erumpent, black, and without "hairy" covering. It was found during inoculation studies that, under certain conditions, erumpent pycnidia were formed on infected apples. The factors responsible for the development of the two types of fruiting bodies will be discussed later.

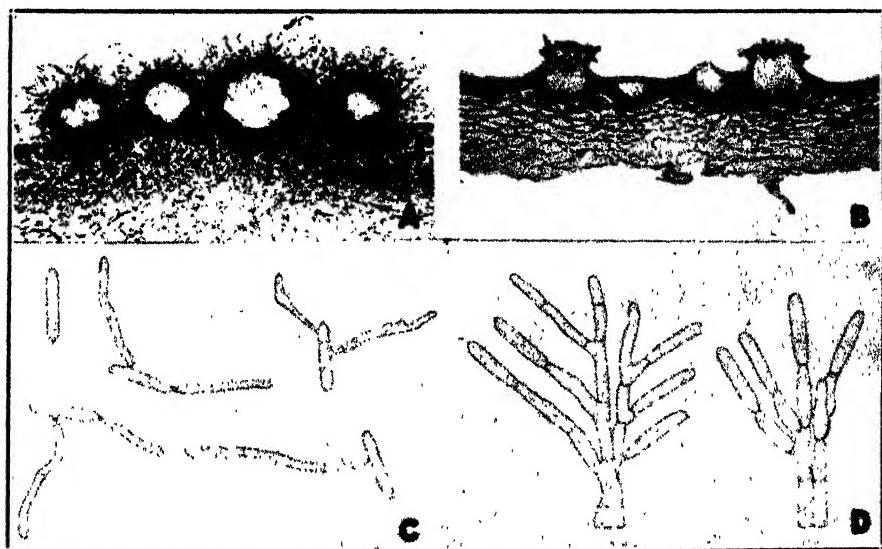


FIG. 2. A. Microtome section of pycnidia produced on potato-dextrose agar. B. Section of pycnidia formed on McIntosh apple. C. Germinated conidia of the pathogen. D. Conidia and conidiophores.

EFFECT OF DEXTROSE ON THE GROWTH AND REPRODUCTION  
OF THE PATHOGEN

The fungus did not grow or reproduce well on potato-agar without sugar. On test-tube slants, hyphae of about 2 cm. in length developed before growth stopped, and only a few immature pycnidia were formed. When dextrose was added to the medium at concentrations of 1, 2, or 5 per cent, thick, black mycelial mats developed within 10 days at room temperature (about 20° C.). The hyphae in contact with the agar at first were deep green, but later became black. The aerial hyphae were grey.

Drops of a black watery substance formed on many of the pycnidia, particularly on the slants containing 2 and 5 per cent dextrose. In others, conidia oozed from the fruiting bodies and formed in greyish masses at their apices.

When the dextrose content of the medium was increased to 10 per cent the mycelial mats were not so thick as those on media with the lower sugar concentrations. There were also fewer aerial hyphae and the pycnidia, although numerous, were small and immature.

OPTIMUM TEMPERATURE FOR THE GROWTH OF THE PATHOGEN

The optimum temperature for the growth of the fungus was determined from 2 series of tests. In one, the organism was grown on 1 per cent potato-dextrose agar in Petri dishes, the dishes being placed in duplicate in temperatures, varying at 3-degree intervals, from 3° to 30° C.

In another test McIntosh apples were inoculated and placed in duplicate at the same temperatures as the agar cultures. Measurements of the lesions on the fruits and the diameters of the mycelial growths on the plates were made at the end of 8 and 14 days.

The optimum temperature for the growth of the pathogen, as determined from both tests, was found to be near 21° C.

Effect of Temperature on the Production of the Pycnidia

The cultures used in the previous test were removed from the constant-temperature chambers at the end of 21 days and left at room temperature (about 20° C.). In all cases the colonies continued to grow; in time, they completely covered the surface of the agar in the Petri dishes. Later, pycnidia developed abundantly on the mycelium formed at temperatures below 18° C. Only a few pycnidia developed on the mycelium formed at room temperature or on that produced in temperatures above 18° C.

These studies indicate that the optimum temperature for the formation of the pycnidia is lower than it is for vegetative growth.

PATHOGENICITY STUDIES

The pathogenicity of the fungus was determined by inoculating 2 or more fruits from each of 25 varieties of apples. The inoculations were made by cutting a V-shape notch about  $\frac{1}{4}$  in. deep in each fruit with a flamed scalpel

and the insertion of a small amount of mycelium taken from an agar culture. After sealing the notch with a gummed paper the apples were placed in glass containers and held at 15° C. for thirty days, when all of the inoculated fruits were found to be severely infected.

The color and size of the pycnidia as well as the abundance of fruiting of the pathogen varied greatly on the apples used in the test. On the variety Arkansas no pyrenidia were formed (Fig. 3, A). On Golden Delicious, Jonathan, Northern Spy (Fig. 3, B), and Turley only a few black, partially-erumpent, pyrenidia formed at or near the lenticels. With Duchess of Oldenburg (Fig. 3, D), Fameuse, McIntosh, Macoun, Milton, and Wealthy numerous black, partially-erumpent, pyrenidia formed on a large part of the apple. On Carleton, Cortland, Gano, Hubbardston, Lobo, Mother, Northwestern Greening (Fig. 3, C), Red Delicious, Red Canada, Rhode Island Greening, Roxbury Russet, Smokehouse, Tolman Sweet, and Twenty Ounce, numerous greyish-erumpent fruiting bodies developed. In all but 2

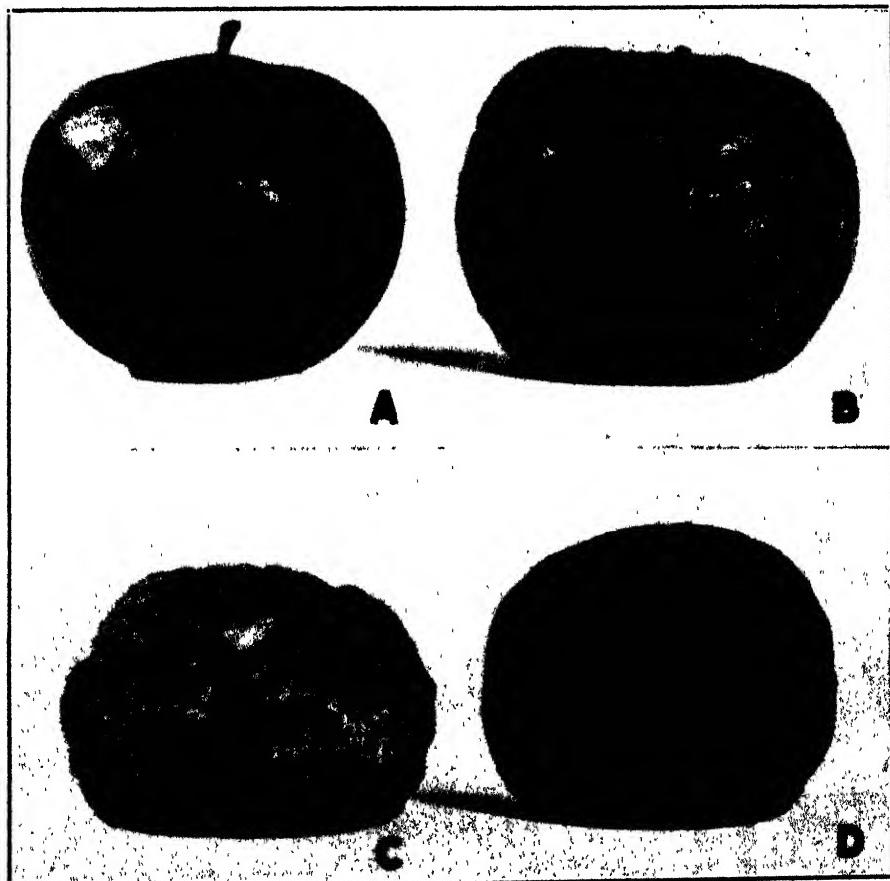


FIG. 3. The formation of pyrenidia on four different apple varieties under the same conditions of temperature and relative humidity. A. Arkansas. B. Northern Spy. C. Northwestern Greening. D. Duchess of Oldenburg.

instances the symptoms were the same on both fruits in each of the varieties inoculated.

As the tissue of the fruits became infected the skin on the red varieties changed to a vinaceous-fawn and appeared more waxy than normal. The skins of the yellow and green varieties changed to an ochraceous-buff and a Dresden-brown, respectively. The surfaces of the lesions were dull rather than waxy. At temperatures above 21° C. the skins on the red varieties changed to a dull brown instead of fawn.

#### Factors Determining the Type of Pyrenidia Produced

The number and the kind of pyrenidia formed on the inoculated fruits of the 25 apple varieties varied considerably. It was noted, however, that, with the exception of Cortland, the 15 varieties on which the erumpent pyrenidia developed were of the late fall or winter sorts all of which have thick skins. Nonerumpent pyrenidia developed in all cases on the fruits of the fall varieties that have thin skins.

Later investigations showed that, under certain conditions, those fruits with thin skins lost sufficient moisture during infection and that only nonerumpent pyrenidia formed on the surface of the apples.

In several tests where fruits from the thin-skin varieties were inoculated and held in relative humidities (usually above 60 per cent) high enough to prevent excessive loss of moisture from the fruits, erumpent pyrenidia formed. At lower humidities the nonerumpent fruiting bodies developed.

Apples with thick skins usually retained sufficient moisture for the production of the erumpent pyrenidia. However, when the humidity was low enough to cause sufficient loss of moisture (in one test it was 10 per cent with Baldwin apples), the nonerumpent fruiting bodies developed.

Only as it affects the loss of moisture from infected fruits does temperature within the range of 3-21° C. influence the type of pyrenidia formed. In no case, either in culture or on apple fruits, were pyrenidia produced by the pathogen in temperatures above 24° C.

Under common apple-storage conditions one may expect to find both the erumpent and nonerumpent types of pyrenidia on infected fruits.

#### The Conidia as Inoculum

The conidia were shown to serve as inoculum when viable conidia of the pathogen were atomized on the uninjured surfaces of McIntosh and Golden Delicious apples, held in moist chambers at 15° C. At the end of 14 days the McIntosh fruits were diseased, and later, fruiting bodies of the pathogen developed on the lesions. The Golden Delicious apples remained unaffected. Where the skins of the fruits from both varieties were punctured before inoculation, with a sterile needle, the fruits of both varieties became infected.

#### Infected Apple Tissue as Inoculum

Tests showed that when infected apples touched healthy fruits they were sources for spread of the fungus under storage conditions. Brown

lesions developed on McIntosh apples from which the pathogen was isolated, when pieces of infected apple tissue were left on the uninjured skins of the fruits under moist conditions. It is not known whether the fungus will penetrate the uninjured epidermis of the thick skinned varieties.

#### Twig Inoculations

Attempts to obtain infection on one-year-old and current-season growth of Rhode Island Greening apple twigs, by inserting bits of the mycelium into cuts in the bark, were unsuccessful. The inoculations were made in the greenhouse and the cuts were covered with wax to prevent drying during the test period.

#### IDENTIFICATION OF THE PATHOGEN

For identification of the pathogen parts of the original apples containing fruiting bodies of the fungus were sent to David H. Linder at Harvard University. There it was identified as *Trichoseptoria fructigena* Maublanc.<sup>2</sup> He pointed out that this was the first report of the finding of a species of this genus in America. The genus *Trichoseptoria*<sup>3</sup> differs from *Septoria* in that the pyrenidia formed by species of the first genus have a hairy covering, while those of the latter do not.

Maublanc described the pathogen and the symptom of the disease as follows (translation) :

Spots somewhat large, depressed, pale reddish yellow. Pyrenidia subepidermal becoming erumpent and then superficial; separate, becoming wavy, confluent, with a twisted, hyaline, hairy, covering. Spores cylindrical, curved, both ends obtuse; granular, with a large central oil drop; rarely 3-guttulate and indistinctly 2 septate, hyaline, 18–23 × 3–3.5  $\mu$ . Conidiophores unequal in length, simple or basally branched. In mature fruits of *Pyrus malus* and *Cydonia vulgaris* in France.

The development of the pyrenidia and measurements for the conidia of Maublanc's fungus closely agree with those made on the fungus under observation. The conidia measured were 17.0–23.8  $\mu$  long by 2.3–3.4  $\mu$  wide. The curved spores, illustrated and described by Maublanc, were observed within the pyrenidia only. Conidia liberated from the fruiting bodies were found to be straight, rarely slightly curved and were pointed at the basal end and not obtuse on both ends (Fig. 2, C). Neither germinated nor ungerminated septate conidia were observed by the writer and it was found that the conidiophores (Fig. 2, C) were much more complex than described or illustrated by Maublanc.

With the exception of these few differences the fungus studied by the writer closely resembles *Trichoseptoria fructigena* Maublanc and is considered to be the same.

#### SUMMARY

A fungus causing a soft rot of McIntosh apples was obtained in pure culture by the writer. It was taken from diseased fruits collected by H. H.

<sup>2</sup> Maublanc, M. A. *Trichoseptoria fructigena*, nov. sp. Bul. Myc. Soc. France 21: 95–97. 1905.

<sup>3</sup> Bender, Harold B. *Trichoseptoria*. The Fungi Imperfeci: Order Sphaeropsidales, p. 7. 1934.

Whetzel. David Linder identified the organism as *Trichoseptoria fructigena* Maublanc.

So far as the writer has been able to determine this is the first report of the finding of this pathogen in North America.

The pathogenicity of the fungus was tested on the fruits of 25 varieties of apples and the signs of the pathogen and the symptoms of the disease were studied and described.

The pathogen grows and reproduces well on 1 per cent potato-dextrose agar. The optimum temperature for growth, both on agar and on apple fruits, is about 21° C. The optimum temperature for pycnidial formation is believed to be somewhat lower.

The effect of temperature and relative humidity on the formation of the pycnidia is discussed.

The conidia of the pathogen and infected apple fruits may serve as inoculum.

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## ETHYL MERCURY IODIDE—AN EFFECTIVE FUNGICIDE AND NEMACIDE

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(Accepted for publication October 28, 1939)

Experience over a period of years with various chemical methods of damping-off control has emphasized the need for some soil disinfectant that will control both pre- and post-emergence damping-off, and remain effective over a period of several months. Particularly is this protection needed for seeds requiring a long germination period and seedlings especially sensitive to soil-borne damping-off fungi. The following representative experiments indicate such surprisingly efficient fungicidal and nemacidal qualities in a product known as DuBay 1155-HH<sup>1</sup> that we are led to believe it meets the above requirements.

Six half flats, 3"×9"×18", were filled with a mixture of  $\frac{1}{2}$  loam,  $\frac{1}{4}$  compost and  $\frac{1}{4}$  peat (Fig. 1). Two of these were left nontreated, two were treated with DuBay 1155-HH, at the rate of 1½ grams per square foot,<sup>2</sup> and the soil of the 2 remaining flats was autoclaved at 30 lbs. pressure for

<sup>1</sup> DuBay 1155—HH was obtained from Bayer-Semesan Company, Inc., and contains as the toxic ingredient 5 per cent ethyl mercury iodide.

<sup>2</sup> When treating soil, DuBay 1155-HH should first be thoroughly mixed with about 30 to 50 times its weight of dry soil or sand. This concentrated mixture is then spread over the surface, first in one direction and then at right angles, thus securing a uniform covering. A dibble may be used to thoroughly mix this concentrated mixture with the rest of the soil. It is necessary to allow a waiting period of 4 to 7 days, the time varying according to strength of treatment, amount of organic material in the soil and type of seed planted. Great care should be taken to weigh out the proper quantity of DuBay 1155-HH for the medium to be treated. If too much is used root injury is very likely to occur, and may easily prove fatal to the plant. One half gram per square foot or less is sufficient for disinfecting river sand.



FIG. 1. Effect of soil treatments on seedling emergence and growth. The same soil mixture was used in all flats. A. Soil autoclaved one hour at 30 pounds' pressure. B. Soil treated with DuBay 1155-HH, the same as D. Note larger size of leaves and plants in chemically treated soil. C. Nontreated control. D. Treated with DuBay 1155-HH at the rate of 1.5 g. per sq. ft. Note poor emergence and survival of stocks, phlox, violas and asters in nontreated control. *The seeds were planted the same way in all flats.*

1 hr. on December 8, 1938. Six days later strips of stocks, *Phlox drummondii*, *Viola cornuta*, White Perfection, and asters, were planted in each flat. Seedlings emerged first in the treated flats, next in the autoclaved soil, and finally, about a week later, in the control. The emergence data are summarized in table 1.

The post-emergence damping-off in the control flats occurred soon after seedling emergence. From 5 to 14 times more seedlings emerged and survived in the treated flats than in the control. Growth was more vigorous than in either autoclaved soil or control. This difference was especially marked about 3 to 4 weeks after emergence, when the photographs shown in figure 1 were taken. In spite of the greater space per plant in the control and in the case of the stocks and phlox in the autoclaved soil as well, the individual plants in the treated soil had more and larger leaves, were much darker green, and bore heavier stems. The stimulation noted after treating soil can hardly be due then solely to suppression of the fungus and resultant

TABLE 1.—Effect of steam sterilization and soil treatment on seedling emergence as shown by total number of seedlings surviving after different treatments

Variety seed	Control			Autoclaved 30 lb. 1-hour			Treated 1½ g. of DuBay 1155-HH per sq. ft.		
	Flat 1	Flat 2	Total surv.	Flat 1	Flat 2	Total	Flat 1	Flat 2	Total
Stocks—100 seeds per flat	12-0 <sup>a</sup>	23-0	35	64-0	22-0	86	90-0	60-0	150
Phlox—300 seeds per flat	5-0	6-0	11	12-0	13-0	25	35-0	47-0	82
Viola—200 seeds per flat	40-30	51-38	23	165-0	156-0	321	172-0	152-0	324
Asters—200 seeds per flat	14-10	12-7	9	47-0	27-0	74	43-0	27-0	70

<sup>a</sup> Figures to left in each column indicate number of seedlings emerging; those to the right, number that damped off after emergence.

healthier root system; it must be due to some effect of the chemical on either the soil or directly on the plant.

In view of the strong fungicidal properties shown by DuBay 1155-HH in numerous experiments, of which the recent one above reported is typical, the following test of its value as a nemacide was made.

On March 31, 1938, a flat of potting-up soil fairly high in organic content was mixed with five 4-inch pots of soil so heavily infested with nematodes that the *Convolvulus cneorum* plants were almost dead. Roots bearing nematode knots also were included. This soil was then divided into 2 parts and placed in half flats, one of which was treated with DuBay 1155-HH at the rate of 2 g. per sq. ft. and the other was left nontreated. Two hundred seeds of Marglobe tomato were sown in each flat on April 13, 1938. Germination was excellent in both flats. Examination of the plants on May 28, 1938, showed 158 infested in the nontreated control. Most of these plants had the roots completely covered with knots, some bore only a few knots, and 2 plants apparently had escaped infestation. All of the 145 plants in the treated flat were completely free of root knots and were about a third larger than the control plants.

Of each group 24 plants were transplanted into ordinary nontreated soil on May 28, 1938. On July 20, 1938, all the plants from the treated soil were free of root knots, while most of the new roots formed on control plants were infested. Periodic examination of the plants was made and, by October 12th, most of the plants in the control group were dead from the severe infestation. One exceptional plant bore only 8 very small knots. Three of the plants from the treated soil had a few small knots, indicating that they recently had become infested by drainage from the nontreated control plants next to them. The other plants from treated soil were all clean. The striking difference in roots of these 2 groups of plants is shown in figure 2.

The above encouraging results led to the following experiment with cuttings, apparently free of knots, which had been rooted in ordinary non-



FIG. 2. A. Tomato plants free of root knot after growing in treated soil. B. Those grown in nontreated soil. DuBay 1155-HH was used at the rate of 2 grams per sq. ft. for soil treatment.

treated sand. These were divided into 3 groups and potted up in non-treated, infested soil; infested soil, treated with DuBay 1155-HH at the rate of 2 g. per sq. ft.; and infested soil, treated at the rate of 3 g. per sq. ft. The soils were treated on June 3, 1938, and the cuttings were transplanted to pots on June 18, 1938.

The results as determined by examination on October 13, 1938, are summarized in table 2. No control was run for *Streptosolen* or *Bouvardia* because these species are so susceptible to nematode attack as to show knots, even when the nematode population in the soil is very low. Complete control by treatments of both 2 and 3 g. per sq. ft. was obtained in all, except 3 plants of *Bouvardia humboldti*. The plants in treated soil were transplanted to 4-inch pots on October 15, 1938, containing soil treated at the rate of 2 g. per sq. ft. All were growing very vigorously and the roots were completely free of root knot when examined on December 23, 1938.

TABLE 2.—Summary of results obtained with DuBay 1155-HH applied at indicated strengths to soil heavily infested with nematodes. Plants examined October 15, 1938

Species	Control	2 g. sq. ft.	3 g. sq. ft.
1. <i>Buddleia asiatica</i>	All 12 plants infested. Only about $\frac{1}{2}$ size of those in treated soil	12 plants free of root knots. Very vigorous	12 plants free of root knots. Very vigorous
2. <i>Abelia floribunda</i>	All 13 plants heavily infested	All 12 plants free of root knots	All 9 plants free of root knots
3. <i>Streptosolen jamesonii</i>	No control	All 18 plants free of root knots	All 18 plants free of root knots
4. <i>Ruellia macrantha</i>	All 9 plants heavily infested	All 17 plants free of root knots	All 15 plants free of root knots
5. <i>Bouvardia humboldti</i>	No control	3 plants infested; 2 free of knots	All 5 plants free of knots
6. <i>Bouvardia Coral Gem</i>	No control	All 4 plants free of knots	All 6 plants free of knots

The complete eradication of nematodes from heavily infested potting soil effected by DuBay 1155-HH, in these experiments, has led to large scale commercial treatments of potting soil and tests of its value as a field disinfectant. Experiments testing its value in the control of damping-off in citrus and particularly sensitive species of ornamentals also are in progress. Reports on the results of these tests will be submitted later.

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#### A MOSAIC DISEASE OF RAPE AND OTHER CULTIVATED CRUCIFERS IN CHINA

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(Accepted for publication Nov. 24, 1939)

A mosaic disease on rape and various other cultivated crucifers is widespread in different districts in China, often causing considerable losses. To determine whether it is identical with any of the virus diseases of crucifers reported from other countries, observations and experiments have been made since 1937 on symptoms, host range, and properties of the virus. The results so far obtained are presented in this paper.

The symptoms of the disease, both in the field and in the greenhouse, are somewhat similar to those of the turnip mosaic on Long Island, New York, as described by Tompkins (4). The infection manifests itself in the beginning as a systemic, conspicuous clearing of veins, usually commencing at or near the base of the leaf and gradually spreading over the entire leaf. Generally, this stage may last for 2 to 3 weeks. In certain cases, particularly under higher temperatures, clearing is soon replaced by vein banding. Dur-

<sup>1</sup> The writers are grateful to Dr. C. M. Tompkins of the California Agricultural Experiment Station, U. S. A., for his kindness in furnishing seed of certain crucifers used in his virus work.

ing the later stages of infection, numerous, raised or non-raised, dark-green islands of irregular outline appear in the chlorotic area between the veins, giving rise to a mottled appearance (Fig. 1, A). All the above-mentioned stages of infection are often accompanied by curvature of the midrib and distortion of the leaf blade. Low temperatures favor the expression of symptoms, and temperatures higher than 20° C. usually induce their masking. Plants infected early are usually severely stunted and often killed, but those infected late in their development are stunted only slightly or not at all.

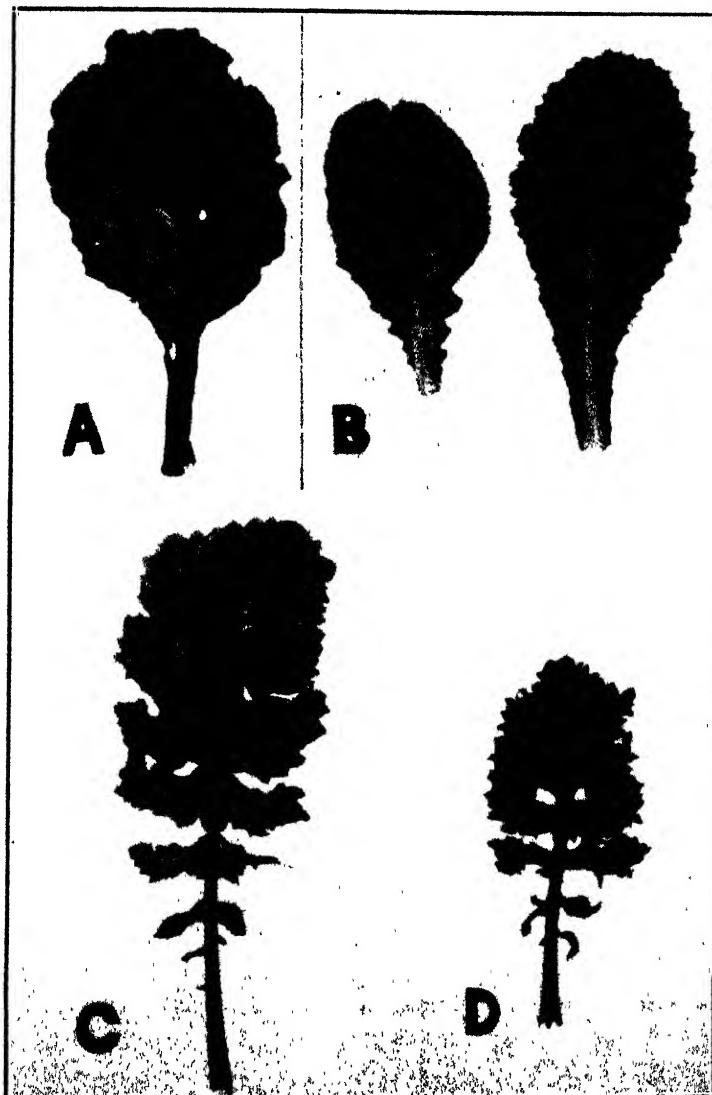


FIG. 1. Symptoms produced by the rape-mosaic virus on leaves of certain crucifers by artificial inoculation in the greenhouse. A. Mottling on rape. B. Vein banding on Chinese cabbage, with noninoculated leaf at right. C. Late stage of vein clearing on turnip. D. Vein banding on turnip.

Attempts to transmit the disease to rape seedlings in the greenhouse by means of rubbing the leaves with expressed juice from diseased plants were unsuccessful. When powdered carborundum (3) was added as an abrasive, infection was obtained in a low percentage of cases. The incubation period ranged from 18 to 26 days. In experiments on insect transmission, only the green peach aphid (*Myzus persicae* Sulzer) was used. Approximately 50 per cent infection was obtained, with the incubation period ranging from 15 to 24 days. Field trials on seed transmission failed to yield positive evidence.

In addition to rape, Chinese cabbage (*Brassica chinensis* L.), turnip (*B. rapa* L.), leaf mustard (*B. juncea* Coss.), and Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey) were found infected with the same virus in nature, the symptoms being identical with those shown by inoculating rape with expressed juices. The results of mechanically inoculating certain cultivated crucifers in the greenhouse are summarized in table 1.

TABLE 1.—*Susceptibility of certain cultivated crucifers to the rape-mosaic virus as determined by artificial inoculation in greenhouse*

Species and common name	Horticultural variety	Number plants inoculated	Number plants infected	Average incubation period, in days
<i>Brassica chinensis</i> L. (pak-choi)	Local (large type) Local (small type)	54 54	5 0	29
<i>B. pe-tsai</i> Bailey (pe-tsai) <i>B. oleracea</i> L. var. <i>caperata</i> L. (cabbage)	Wong Bok Local Winter Colma	28 46 21	3 0 0	14
<i>B. oleracea</i> L. var. <i>caulorapa</i> DC. (kohlrabi)	Local	48	0	
<i>B. oleracea</i> L. var. <i>botrytis</i> L. (cauliflower)	February	53	0	
<i>B. rapa</i> L. (turnip)	Local Purple Top White Globe	54 34	11 10	22 14
<i>B. juncea</i> Coss. (leaf mustard)	Local	52	5	27
<i>Raphanus sativus</i> L. (radish)	White Icicle	31	0	
<i>R. sativus</i> L. var. <i>longipinnatus</i> Bailey (Chinese radish)	Local (round type) Local (spring type)	54 54	24 10	26 25

Purple-top White-globe turnip was shown by Tompkins and Thomas (5) to be the best host for separating the viruses of crucifers that they studied. In our study with this particular host, as well as the local variety of turnip, the symptoms produced by the rape-mosaic virus are characterized by systemic, coarse vein clearing (Fig. 1, C), ruffling of the leaf, followed by coarse



FIG. 2. Symptoms produced by the rape-mosaic virus on leaf mustard under natural conditions, showing rugosity and distortion of leaf and stunting of plant. A. Healthy plant. B. Infected plant.

mottling, with raised, dark-green areas interspersed (Fig. 1, D). On Chinese cabbage, a systemic, coarse, and yellowish type of vein banding (Fig. 1, B)

TABLE 2.—*Properties of the rape-mosaic virus*

Longevity in vitro, 11°–13° C.					
Time aged, in hours	Number plants inoculated	Number plants infected	Time aged, in hours	Number plants inoculated	Number plants infected
0	18	11	96	18	2
4	18	6	120	18	3
8	18	3	144	18	0
12	17	2	168	18	0
24	18	4	192	18	0
48	18	6	216	18	0
72	18	2	240	18	0
Tolerance to dilution					
Dilution	Number plants inoculated	Number plants infected	Dilution	Number plants inoculated	Number plants infected
0	36	4	1: 3000	36	2
1: 10	36	5	1: 4000	36	1
1: 100	36	5	1: 6000	36	3
1: 500	36	4	1: 7000	36	0
1: 1000	36	1	1: 8000	36	0
Thermal inactivation point					
Temperature, in degrees C., for 10 min.	Number plants inoculated	Number plants infected	Temperature, in degrees C., for 10 min.	Number plants inoculated	Number plants infected
Control	9	6	65	9	0
50	9	3	70	9	0
55	9	2	75	9	0
60	9	3			

is predominant, often accompanied by the curvature of the midrib and the stunting of the plant. On leaf mustard, the rape-mosaic virus causes pronounced rugosity, distortion of the leaf, and severe stunting of the plant (Fig. 2). Symptoms on Chinese radish are similar to those described for rape. Cabbage and cauliflower, the other two differential hosts used by Tompkins and Thomas (5), failed to show any sign of infection after repeated artificial inoculations.

In the study on the properties of the virus, the local variety of rape was used as the test host. The virus in extracts from rape was inactivated at the end of 6 days after storage at a temperature of 11° to 13° C. The thermal inactivation point for a 10-minute exposure in a water bath was between 60° and 65° C. A tolerance to dilution of 1 to 6,000 was established. The results of different trials are summarized in table 2.

Although similarities in symptoms are found between the rape mosaic and the turnip mosaic (4), the host range serves as a chief differential character. The rape-mosaic virus is unable to infect cabbage and cauliflower. On the other hand, rape is not listed as a host of the turnip mosaic. Further search of the literature reveals only two cases, one from Germany (2) and the other from New Zealand (1), where rape is recorded as being susceptible to virus disease in nature. The descriptions given in both cases, however, are not in agreement with ours either in respect to symptoms or host range. On the basis of the information hitherto available, therefore, our rape mosaic appears to be an undescribed one.

#### SUMMARY

A description is given of a mosaic disease occurring on rape and 4 other cultivated crucifers in China. Characteristic symptoms consist of vein clearing in the initial stage of infection, followed by vein banding and conspicuous mottling with dark-green areas. Under greenhouse conditions, the virus is transmissible by the green peach aphid and also by mechanical rubbing, with powdered carborundum added as an abrasive. The virus remains infective after a 10-minute exposure to a temperature of 60° C., after storage for 5 days at 11° to 13° C., and after being diluted to 1 to 6,000.

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## PHYTOPATHOLOGICAL NOTES

*Tomato Fruit Pox.*—An abnormal condition affecting green and ripe tomatoes was found in southwestern Texas in 1937, and was mentioned under a tentative name of fruit mottle.<sup>1</sup> The first symptom on green fruits consists of many conspicuous, abnormally dark green dots scattered over the fruit surface. Where numerous, these dots give the fruits a mottled appearance (Fig. 1, A). The dark green spots vary from a very small speck to about 3 mm. in diameter, and are round, elongated, or irregular. Although often distributed at random in the fruit surface, they are more frequently found along the meridians extending from the stylar scar to the pedicel (Fig. 1, B). Several of the spots may coalesce and form a streak with its long axis usually oriented meridionally. Later, many of the dark green spots become sunken as pits or pox with ruptured surface tissues (Fig. 1, C, D). This abnormality was named "tomato fruit pox" because of the pock-like marks in the fruit peel.<sup>2</sup>

The dark green mottle spots are found on fruits of all ages, while most of the pox marks are found on green-wrap, pink, and ripe fruits. The change from the unbroken, dark green spots to the pitted, pox stage occurs in a few days, according to observations of many fruits in the laboratories. Presumably, this change may occur also during transit and storage. As fruits turn pink and red, the dark green spots remain green or turn yellow, while the pox may cork over and appear as an abnormally large lenticel upon the surface of the fruit. Besides making tomatoes unmarketable as first grade fruits, the pox spots may serve as points of infection for fungi and bacteria.

The dark green spots and pox marks appear to be the only symptoms of the trouble, since no other correlated symptoms have yet been found in the plants bearing the affected fruits. Usually all or nearly all of the fruits of affected plants show pox symptoms. More than 90 per cent of the plants were found to be affected in some fields, whereas only a trace of diseased fruits was found in other fields in the same season. Since its discovery, tomato fruit pox has been observed in five seasons of spring and fall crops (1937 to 1939) in the Winter Garden region of Texas. It caused serious economic loss there in the fall of 1938. In some fields, about 10 per cent of the harvested fruit was discarded because of pox injury. Examination of the green-wrap tomatoes at a shipping platform in that season revealed pox symptoms on 10 to 20 per cent of the fruits of some lots. A few affected fruits were found near Jacksonville, Texas, in 1938 and 1939.

The geographic range of tomato fruit pox is partly known. In the winter of 1938-39, the senior writer found pox-affected tomatoes on the

<sup>1</sup> Young, P. A., G. E. Altstatt, and A. J. Harrison. Plant disease survey of southwest Texas. U.S.D.A. Plant Dis. Rptr. 22: 8. 1938.

<sup>2</sup> Ivanoff, S. S. Tomato fruit "pox." Texas Agr. Exp. Station Fifty-first Annual Report, p. 261, 1938.

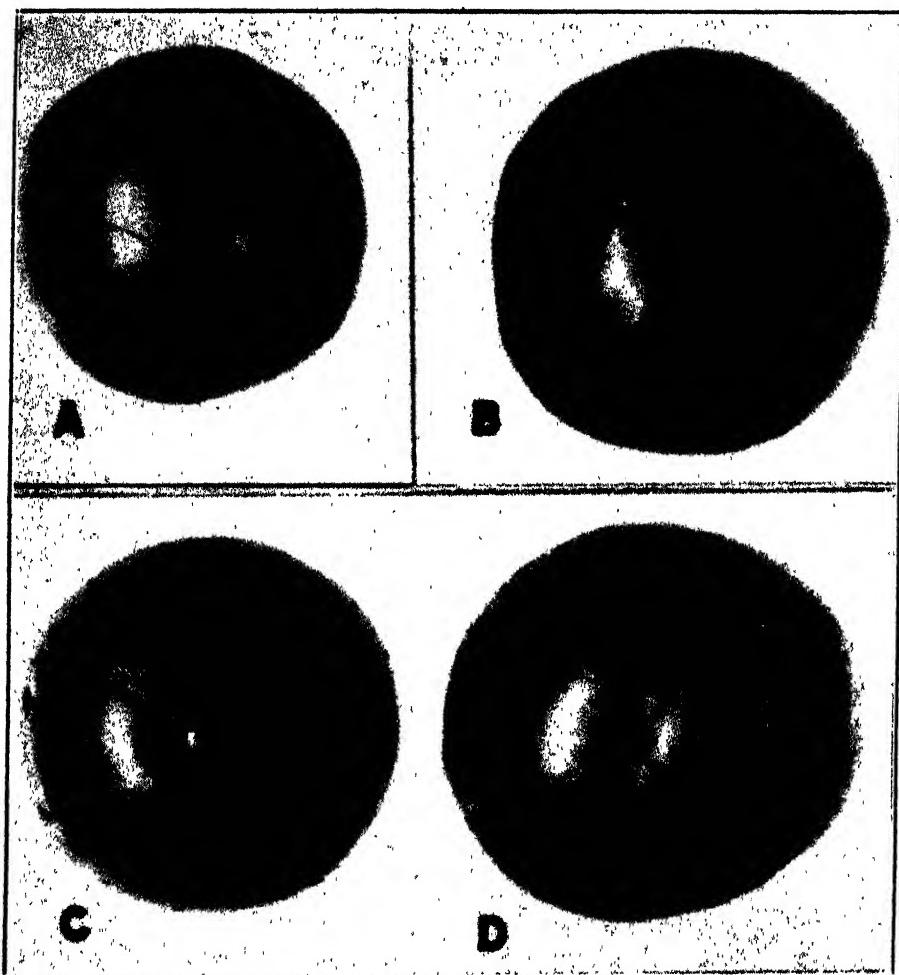


FIG. 1. Tomato fruit pox. A. Green tomato fruit showing numerous small, dark green areas, the early symptoms of the disease. B. Green fruit showing the same areas grouped mainly along the meridians extending from the stylar scar to the pedicel. C and D. Green fruits showing many pits or pox at places where the dark green spots had been. Photographs by L. R. Hawthorn.

markets in Washington, D. C., Virginia, Georgia, and Alabama on fruit shipped in (according to information secured from wholesale dealers) from Florida, Texas, Mexico, Puerto Rico, and Cuba. In the summer of 1939, fruit pox was found to a slight extent affecting Pritchard tomatoes in a few fields in Illinois and Wisconsin.

As to varietal susceptibility, fruit pox in Texas was found most frequently affecting the Pritchard and Stokesdale varieties of tomato, but Marglobe, Rutgers, Bonny Best, Earliana, Summerset, and Globelle also were affected.

The cause of tomato fruit pox is unknown, as far as the writers are aware. No bacteria or fungi have been isolated from affected fruits despite

many attempts to culture a pathogen. Microscopic examinations of affected tissues have not revealed any microorganisms. Apparently, the condition is not hereditary, as it affects many varieties, is found to some extent in many regions, and seed from pox-affected fruits produced plants that bore only normal fruit. It causes serious economic loss of quality in tomato fruit and, therefore, merits further study. The writers would appreciate receiving information about the occurrence of this abnormality in other regions.—  
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*Seedling Stem Blight of Soybean Caused by Glomerella glycines*.—Although Lehman and Wolf,<sup>1</sup> in their paper on soybean anthracnose, stated that soybean plants in all stages of development are subject to infection by *Glomerella glycines* (Hori) Lehman and Wolf, their description of the disease is limited to the effect of the parasite on mature plants. In soybean fields in Szechuen Province, West China, it has been observed frequently that seedlings sometimes are killed by the anthracnose fungus soon after emergence. Infection first appears on the cotyledon as darkened cankers and gradually extends downward to the hypocotyl (Fig. 1). The young stem is rotted and after a short time collapses. In case the infection fails to spread as described, the primary leaves, although in contact with the

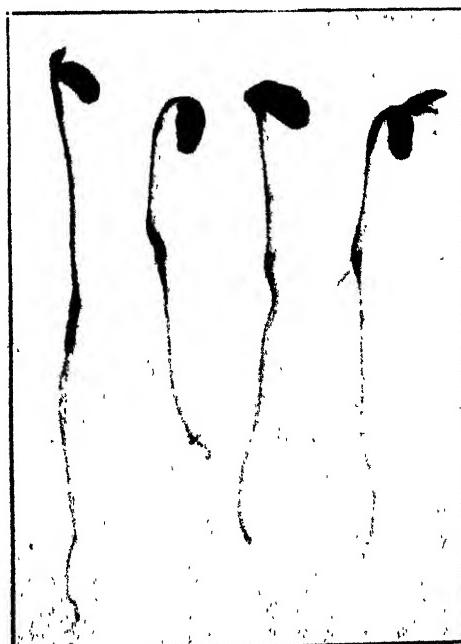


FIG. 1. *Glomerella glycines* on the cotyledons and hypocotyls of soybean seedlings.

<sup>1</sup> Lehman, S. G., and Frederick A. Wolf. Soy-bean anthracnose. Jour. Agr. Res. [U.S.] 33: 381-390. 1926.

cotyledons, will not be affected. During wet weather, setose, black acervuli of the fungus are produced abundantly on the lesion.

In a study of parasitism on seedlings, experiments were made with a strain of monosporous origin, designated as 2a. Artificial inoculations in sterilized and nonsterilized soils were made in 3 ways: (1) By soaking the seeds for one hour in a spore suspension of the fungus; (2) by pouring a spore suspension over the soil surface; (3) by mixing the fungus culture with the soil. For each treatment, 100 seeds of the locally used variety of soybean were planted. As shown in table 1, the fungus is able to kill the seedlings in either pre- or post-emerging stages. When seeds were soaked in spore suspension, half or more were killed before emergence and the rest after emergence, so that complete failure of the plants resulted, regardless of whether the soil was sterilized or not. When the inoculum was mixed directly with the soil, the percentage of seedling survival appeared to be higher in nonsterilized than in sterilized soil. Apparently this might be the result of antibiotic action of other soil organisms present. Another series of experiments in which a mixture of 5 strains of the fungus was used gave similar results.

TABLE 1.—*Results of inoculating soybeans with strain 2a of *Glomerella glycinea**<sup>a</sup>

Treatment	Sterilized soil			Non sterilized soil		
	No. plants emerged	Plants killed after emergence		No. plants emerged	Plants killed after emergence	
		No.	Per cent		No.	Per cent
Seeds soaked in spore suspension	36	36	100	41	41	100
Spore suspension poured over soil	66	62	94	70	27	38
Soil mixed with fungus culture	75	71	95	50	36	72
Check	96	3	3	77	7	9

<sup>a</sup> Results are based on quadruplicate pots with a total of 100 seeds in each treatment.

The mycelium of the fungus, either inside the seeds or surviving in the soil, serves as the primary source of infection. Cultures often have been obtained by ordinary isolation methods from seeds 1 to 2 years old. Under experimental conditions, potted soils that were artificially inoculated with cultures from sterilized bean pods in late fall provided active inoculum until the following spring. This has been shown by the appearance of seedlings with typical lesions and the lowering of the percentage of germination of soybean seeds.

Conidia of the fungus are short-lived and very susceptible to drying. The following experiment is representative of several showing similar results. Conidia were washed off the matrix of inoculated bean pods with distilled water, such as might occur during rain under natural conditions. After drying on clean glass slides, germination tests of the conidia were made at intervals of 6 hours. Before drying, 94 per cent of the spores

germinated under standardized conditions; after drying for 6 hours, only 7 per cent germinated. No germination was observed after 12 hours. Even when the conidial masses were permitted to remain in the matrix from which they extruded, after being transferred to and dried on slides, they have failed to germinate after 24 hours. Germination after 6, 12, and 18 hours was 35, 5, and 2 per cent, respectively, in contrast to 96 per cent of germination in the control series.

The ascigerous stage of the fungus has not yet been found in this region.—LEE LING, The Szechuen Provincial Agricultural Improvement Institute, Chengtu, China.

*A Leaf Spot of Italian Prune Perpetuated in Budded Stock.*—Most Italian-prune orchards observed by the writer in Idaho in the last few years have shown varying amounts of leaf spot and defoliation; reports from other sources indicate that this condition may have been present for many years. The trouble was especially severe in 1936. For the last 3 summers individual trees have been seriously affected, while mild leaf spotting has appeared on most of the trees observed. Attempted isolations and fungicidal treatments have failed to indicate that the spotting is caused by a parasitic fungus.

The symptoms apparently do not appear until early summer. Circumstantial evidence indicates that the spotting comes on rapidly, and the defoliation occurs quickly if the spotting is severe. Of course, reduction in active leaf surface prevents normal photosynthesis and frequently results in



FIG. 1. A leaf spot of Italian prune. A. Leaf from original tree. B. Leaf of shoot resulting from bud from original tree. C. Enlarged portion of leaf similar to A.

heavy fruit drop or a yield of poor fruit. The spots on the leaves vary in size from very small (1-2 mm.) to large blotches and irregular dead areas (Fig. 1). Shot-holing may occur. An indistinct mottling often accompanies severe leaf spot; in some years it may be the only symptom on certain trees. Some observations show that the leaf spot is more severe on trees near the barn lot, chicken runs, or driveways. Affected trees may show very little or extensive terminal growth. It may be of interest to record also that a similar case of serious leaf spot and defoliation has been observed by the writer in the Milton-Freewater district in Oregon.

In August, 1938, bud wood was collected from a tree affected with leaf spot and from a healthy tree in an orchard in northern Idaho and, in September, bud wood from a tree severely affected with leaf spot in southern Idaho. Buds were set on healthy J. H. Hale and Elberta trees at Moscow and observations made during the 1939 season. Shoots of the buds from the healthy prune tree showed no leaf spotting throughout the summer, while severe leaf spot and some chlorosis developed on all shoots from diseased buds. No symptoms appeared on the peach shoots accompanying the diseased prune branches. Unfortunately, no diseased buds were set on prune stock; therefore, there is yet no evidence that the disease factor is transmissible.

The results indicate that the leaf spot and defoliation symptoms, attended by other undesirable factors, in certain Italian-prune trees of Idaho represent either a virosis or a genetic abnormality. The writer believes, however, that environmental factors play an important, although unknown, rôle in the occurrence of this serious trouble.

Further budding work was begun in an attempt to infect healthy prune trees with leaf-spot buds. Additional data may be available next year, but in the meanwhile the trouble is of sufficient interest to warrant this note.—  
EARLE C. BLODGETT, University of Idaho, Moscow, Idaho.

*A Miniature Root-observation Box.*<sup>1</sup>—For the direct observation of root development and root pathology in soil, glass-sided boxes, such as described by Dean,<sup>2</sup> frequently serve admirably. For detailed work with small plants, where frequent microscopic studies during short periods are required, however, the writer has profitably substituted the much smaller box here described.

This box consists of a U-shape wooden frame with its 3 inner faces grooved to hold 2 wide microscope slide glasses (2 by 3 in.) in parallel planes  $\frac{1}{2}$  in. apart. The frame is constructed from strips of sugar pine or similar lumber smoothed to  $\frac{1}{8}$  by  $\frac{7}{16}$  in., with 2 longitudinal grooves spaced  $\frac{1}{2}$  in. apart on one face. These grooves are  $\frac{1}{16}$  in. wide and  $\frac{1}{8}$  in. deep. From such strips 2 pieces are cut  $3\frac{5}{16}$  in. tall to form the sides of the frame. The bottom is a piece  $2\frac{1}{16}$  in. long, with a small hole for drain-

<sup>1</sup> Published with the approval of the director as Miscellaneous Paper No. 32 of the Pineapple Experiment Station, University of Hawaii.

<sup>2</sup> Dean, A. L. Root-observation boxes. *Phytopath.* 19: 407-412. 1929.

age bored through the center. Before assembling, a  $\frac{7}{8}$  in. length at the bottom end of each side piece is cut away flush with the bottom of the grooves, forming recesses into which ends of the bottom piece fit. The frame is then assembled with glue and brass screws, with the grooves aligned to permit the glasses to be slid in and out. To permit removal of one glass without sliding, the flange beyond the groove to hold this glass may be cut away from the sides of the frame, but not from the bottom. One end of the glass may then be inserted into the bottom groove, the glass pressed into the recesses in the side pieces, and secured in place with small brads thrust into the wood as glass panes are held in a sash. The finished frames, waterproofed by dipping into a clear lacquer, are light and durable.

Such boxes, with glasses in place, are filled with the desired soil or other medium (black sand<sup>3</sup> is particularly favorable for observations of nematodes) and the plant or seed is set close to one glass. They are then held in a slanting position in appropriate boxes or frames that are constructed and disposed to protect roots from light and the soil from overheating while exposing top growth to light. Close attention to watering is naturally required and tops of fast-growing plants may require pruning.

For observation, a box is placed onto the microscope stage and illuminated diagonally from above with a beam of light cooled and focused by a spherical flask of water. For general use a dissecting binocular is advantageous, but, for camera-lucida drawings, photomicrographs, and critical observations, the lower magnifications of a compound microscope are employed.

Such boxes proved distinctly superior to the Petri dishes employed in most of the writer's study of attractiveness of roots for nematodes.<sup>3</sup> Their use has also facilitated unpublished studies on the parasitism and development of other nematodes, and promises to be useful in varied studies of root pathology.—M. B. LINFORD, Pineapple Experiment Station, Honolulu, Hawaii.

#### BOOK REVIEWS

BAWDEN, F. C. *Plant Viruses and Virus Diseases*. 272 p., 37 fig. Price 7 guilders or about \$4. Chronica Botanica Co., Leiden, and G. E. Stechert and Co., New York, 1939.

This publication, prepared by the Virus Physiologist of the Rothamstead Experiment Station, deals primarily with the nature of plant viruses rather than with virus diseases. Considering the enormous advance that has been made in an understanding of the nature of plant viruses during the last six years and the fact that much of the work has been with chemical and physical techniques with which most plant pathologists are not too familiar, this critical summary and interpretation should be welcomed. It is probable that there will be disagreement, on the part of those more familiar with the nature of the virus, with the author's conclusions, if one may judge by his conclusions with respect to problems with which the plant pathologist is more familiar, but this is to be expected.

Chapter I is an introductory survey of the virus problem. It includes a tentative definition of "a virus as an obligately parasitic pathogen with at least one dimension of less than 200 mμ," a history of virus diseases, and theories pertaining to them. In a discussion of nomenclature the author points out the obvious impossibility of adopting a

<sup>3</sup> Linford, M. B. Attractiveness of roots and excised shoot tissues to certain nematodes. *Helminthol. Soc. Wash. Proc.* 6: 11-18. 1939.

system of nomenclature such as that proposed by James Johnson, which is not based upon a classification of the viruses themselves. Chapters II and III are concerned with "Syptomatology" in which the variation in symptoms that may be produced by a single virus, depending upon the stage of the disease, the strain of the virus, the environment, and the genetics of the plant, are pointed out. The use of symptoms, such as local necrotic spotting, in quantitative studies, is discussed. In Chapter III are discussed X-bodies, crystalline plates, crystals, etc., and the relation of virus to these bodies; and internal changes other than intracellular bodies. In Chapters IV is discussed "Transmission and properties in expressed sap" including grafting, mechanical, insect, and seed transmission, and resistance to aging, etc., and effect of enzymes and chemicals. Chapter V is concerned with the "Relationships between viruses and their insect vectors." Chapter VI takes up "Virus strains, mutation, and acquired immunity." After discussing variations, differentiation of strains, and origin of strains, considerable attention is given to acquired immunity. Without defining what is meant by immunity, the author proceeds to discuss two types. The first, in which a plant in the chronic stage of a disease (a so-called recovered ring-spot tobacco plant) fails to repeat the earlier phases of the disease when reinoculated with the same virus into already invaded tissue; the second, that in which a plant in the chronic stage of the disease (for example, tobacco mosaic) fails to develop a second similar disease when inoculated with a slightly different strain of the same virus into solidly invaded or diseased tissue. The first is evidently based on a misconception of the ring-spot disease as the author considers only ring and line patterns as symptoms, disregarding leaf-edge chlorosis and necrosis, general chlorosis of yellow ring-spot plants, and pollen sterility, (all symptoms which follow so-called recovery) as symptoms of the chronic stage. The second type of "acquired immunity" appears to be identical with the first, except that the term is applied to plants in which the chronic symptoms are more obvious and the virus from which the plant is immune is another strain of the one already present. Obviously, a plant that already has a disease in a virulent form and will continue to have it as it grows cannot be immune from the virus causing the disease; and it is well known that the plant will not be immune from other strains of the same virus if unoccupied tissue can be located in which the second virus can multiply. The phenomenon has uses in the grouping of viruses and is of some possible value in protecting plants against more injurious strains of the same virus; but there appear to be no sound grounds for concluding that the plants "have developed an immunity to the disease." It would appear for the present that competition is a sufficient explanation for the phenomenon and that the term protection could well replace immunity.

Chapter VII considers in detail "Serological reactions of plant viruses." The discussion of "Specificity of serological reactions" is of interest in relation to classification of viruses. If antisera are group-specific, then such supposedly unrelated viruses as tobacco-mosaic viruses and cucumber viruses 3 and 4 which, in common with tobacco-mosaic virus, withstand long periods of drying, must be grouped together, a situation that could not be considered under Johnson's proposed system. The discussion of serum absorption experiments would lead one to the conclusion that viruses, not serologically related if tested with their respective antisera, might be proved to be serologically related if tested with a third antiserum. That is if one virus contained antigens a and b and another c and d they would appear to be unrelated, but if a third virus contained antigens b and c it would be related to both groups and consequently all three must be considered related. We might conclude from this that only positive serological reactions can be considered in classification of viruses.

Chapter VIII is a discussion of "Purification of viruses" by chemical means and by high-speed centrifugation. The details of purification methods used with 4 distinct plant viruses are given and the products of purification described. Chapter IX considers the "Properties of purified virus preparations" from the chemical and physical standpoint and Chapter X deals with the "Optical properties of purified virus preparations." The author attempts to keep these discussions on a plane that can be understood by the person not well trained in the technics now being used in the study of viruses. Chapter XI is entitled "The sizes of viruses." Measurement of sedimentation velocities and viscosity and the structure of the virus particles are discussed. In Chapter XII the "Correlation of virus activity with the isolated nucleoproteins" is considered in an attempt to give the evidence for and against the virus being identical with nucleoprotein. Chapter XIII is concerned with the "Physiology of virus diseased plants." Host metabolism and virus movement are considered. In Chapter XIV, "Classification and control," the author discusses the necessity of a knowledge of the virus, its host relations and insect vectors if sound control measures are to be arrived at. Again, a plea is made for a lasting classification and nomenclature of viruses based on the virus itself. General control measures discussed are curative, immune varieties, tolerant varieties, extremely susceptible varieties, protection by noninjurious strains of virus and others. The author is apparently unfamiliar with the progress that has been made in the control of tobacco mosaic by elimi-

nating the use of barn-cured tobacco by workmen, but considers that the first infections are "chiefly from virus present in commercial tobacco or in the soil," sources which have not yet been proved to be of importance.

In the final chapter the origin and multiplication of viruses is discussed. The question as to whether viruses are to be considered living or lifeless molecules cannot, in the author's opinion, be answered until the word life can be accurately defined but this may well come from further studies of viruses. How viruses multiply and how they have arisen are questions that cannot at present be answered.

The plant pathologist who has not been able to follow the voluminous literature on the nature of virus will find this book a very welcome addition to his library, as it gives a clear, concise discussion of most of the literature by one who has been actively engaged in studying the chemistry and physics of the virus.—W. D. VALLEAU.

**ASHBY, HELEN, ERIC ASHBY, HAROLD RICHTER and JOHANNES BÄRNES.** *German-English Botanical Terminology.* 195 p., 10/-—Net in Great Britain—Thomas Murby & Co., London; Max Weg, Leipzig; Nordemann Publishing Co., Inc., New York, \$3.00.

The volume on German-English Botanical Terminology is the third of Murby's German-English terminologies. It presents an introduction to German and English terms used in botany, including plant physiology, ecology, genetics, and plant pathology. A brief survey of botanical science is given in English and German. The manner of presentation consists of short and concise drafts on each subject matter in English on one page and a translation of the same in German on the opposite page. This method makes it very useful for the student. Wherever possible, the German text is primarily a literal translation of the English. In instances where this is not possible, since the same idens are often expressed differently in German and English, the idiomatic translations are given. A thorough study of the book will acquaint the student with the technical terms used by German- and English-speaking botanists.

Three appendices are included. Appendix I gives the English, Latin, and German names of common, wild and cultivated plants, especially of those growing in Europe. Appendix II presents a list of the most important common names of plant diseases. The name, host, and cause are listed in both English and German. In appendix IIIa are given the abbreviations frequently used in German botanical literature along with the English translation. Appendix IIIb gives the abbreviations frequently used in English botanical literature with the German translation.

The method of presentation of the subject matter is excellent. Since the authors of the work are well-qualified botanists, the volume should find a good reception by students in both English- and German-speaking countries.—OTTO A. REINKING, N. Y. State Agr. Exp. Station, Geneva, N. Y.

# REPORT OF THE THIRTY-FIRST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

## THE 1939 COLUMBUS MEETING

The thirty-first annual meeting of The American Phytopathological Society, held in Columbus, Ohio (December 27 to 30, 1939), was one of the most successful and best attended meetings the Society has held. Approximately 325 members were in attendance. Sixty-two new members were elected at Columbus, bringing the active membership roll to 1082, a new high record for membership in the Society.

Nearly 300 attended the Phytopathologists' Dinner at the Neil House, and enjoyed the program arranged by President Orton and his capable coworkers from the Department of Plant Pathology of West Virginia University.

Special conferences were held on plant disease survey, disease resistance in plants, eradicant fungicides, recent studies on fire blight of apples and pears, and laboratory testing of fungicides. The value of cooperation among different branches of plant science was brought out in joint sessions with the American Society of Economic Entomologists, Section G, A. A. A. S., and affiliated Botanical Societies, the Potato Association of America, the Mycological Society, the Association of American Foresters, and the Physiological Section of the Botanical Society of America and the American Society of Plant Physiologists.

The summer meeting will be held in Seattle, Washington, June 17-22, 1940.

### OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1940

#### Officers:

CHARLES CHUPP, President (1 yr.), Cornell University, Ithaca, New York.

J. G. LEACH, Vice-President (1 yr.), West Virginia University, Morgantown, West Virginia.

R. S. KIRBY, Secretary (3 yrs. term expires 1940), Pennsylvania State College, State College, Pennsylvania.

H. A. EDSON, Treasurer of the Society and Business Manager of *PHYTOPATHOLOGY* (3 yrs. term expires 1940), U. S. Department of Agriculture, Washington, D. C.

H. B. HUMPHREY, Editor in Chief of *PHYTOPATHOLOGY* (3 yrs. term expires 1940), U. S. Department of Agriculture, Washington, D. C.

#### Councilors:

H. W. ANDERSON (term expires 1940), University of Illinois, Urbana, Illinois.

J. B. KENDRICK (term expires 1942), University of California, Davis, California.

C. R. ORTON (term expires 1940), West Virginia University, Morgantown, W. Va.

EUBANKS CARSNER (2 yrs. for the Pacific Div.), P. O. Box 31, Riverside, Calif.

GEO. M. ARMSTRONG, (2 yrs. for the Southern Div.), Clemson Agric. College, Clemson, S. C.

#### Representatives:

A. A. A. S. Council (1 yr.), L. M. Massey, C. R. Orton.

*Elector Group V, Division of Biology and Agriculture, National Research Council*  
(terms expire June 30, 1940), E. C. Stakman (H. P. Barss, alternate).

*Tropical Research Foundation* (5 yrs. term expires 1940), L. R. Jones.

*International Union of Biological Sciences*, A. G. Newhall.

*Board of Editors, American Journal of Botany* (3 yrs. term expires 1940), G. W. Keitt.  
*Union of American Biological Societies (and Biological Abstracts)*, H. B. Humphrey

and R. S. Kirby (*ex officio*) H. P. Barss, C. W. Bennett, H. A. Edson, G. W. Keitt.

#### Standing Committees:

*Advisory on Society Activities and Programs*. W. J. Zaumeyer, Chm., F. L. Drayton,  
A. A. Dunlap, J. A. Pinckard, R. K. Voorhees, J. C. Walker, C. E. Yarwood.

*Coordination in Cereal and Vegetable Seed Treatment Research*. M. B. Moore, Chm.,  
W. E. Brentzel, H. T. Cook, F. J. Greaney, II. A. Rodenbiser.

*Donations and Legacies*. E. C. Stakman, Chm., J. G. Brown, N. J. Giddings, N. E. Stevens, R. P. White.

*Extension Work and Relations*. Luther Shaw, Chm., C. C. Allison, R. J. Haskell, G. W. Keitt, R. H. Porter, O. A. Reinking, R. C. Rose, D. R. Sands, W. B. Tisdale.

*Investments*. H. A. Edson, Chm., Charles Brooks, Marvin E. Fowler, J. W. Roberts.

*Necrology*. A. G. Johnson, Chm., M. B. Waite.

*New Memberships and Subscriptions.* R. F. Poole, Chm., J. C. Carter, Kenneth Kadow, R. S. Kirby (*ex officio*), L. D. Leach, R. M. Lindgren.

*Nomenclature and Classification of Plant Viruses.* James Johnson, Chm., C. W. Bennett, Eubanks Carsner, F. O. Holmes, H. H. McKinney, H. H. Thornberry, Freeman Weiss.

*Phytopathological Classics.* H. H. Whetzel, Manager, H. B. Humphrey, Editor.

*Publicity and Public Relations.* C. T. Gregory, Chm., O. C. Boyd, J. H. Jensen, Frank McWhorter, A. G. Newhall, J. A. Pinekard, G. H. Starr, A. J. Ullstrup, G. F. Weber, P. A. Young.

*Standardization of Fungicidal Tests.* S. E. A. McCallan, Chm., R. H. Daines, J. G. Horsfall, K. J. Kadow, J. W. Roberts, C. E. Yarwood, H. C. Young.

#### TEMPORARY COMMITTEES

*Auditing,* E. E. Clayton, Ross W. Davidson.

*Elections,* Max Gardner, Chairman, C. E. Yarwood, P. A. Ark.

*Resolutions,* G. W. Keitt, Max Gardner, G. H. Coons.

#### REPORTS OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1939

**Report of the Secretary.** The Society year 1939 opened with 1077 members and closed with 1082, a gain of 5 members. At the Columbus meeting 62 new members were elected. Fourteen former members were restored to the active roll during the year. The Society lost 71 members, 17 by resignation, 5 by death, and 49 by suspension for non-payment of dues. Of the full membership, 151 are paid-up life members and 8 are paying \$10.00 per year toward life membership.

The Society's clearing agency was established to facilitate contact between employable plant pathologists and phytopathological openings from individuals or institutions. Applications were received from 33 plant pathologists desiring positions and from 11 prospective employers. Fifty-seven individual applications were sent to prospective employers. Four plant pathologists reported as having obtained positions through contacts started by the agency.

R. S. KIRBY

**Report of the Treasurer.** Statement of accounts for the year ending November 30, 1939.

*Receipts:*

Balance for 1938		\$4296.22
Annual dues:		
1938	\$ 31.90	
1939	2859.08	(\$134.22 life)
1940	1919.00	( 70.00 life)
1941	1.00	
		\$4810.98
Voluntary dues		5.00
Interest on savings account		88.08
Items for other accounts included in checks for dues:		
Sales	2.00	
Subscription	6.50	
Classics	1.25	
Dues, Mycological Society	5.00	14.75
To replace checks returned by bank		9.07
Total receipts		4927.88
		\$9224.10

*Expenditures:*

Member subscriptions transferred to PHYTOPATHOLOGY:

1938	\$1703.30
1939	1000.00

\$2703.30

104.58

Transferred to Sinking Fund (Building and Loan)

Transferred to PHYTOPATHOLOGY for publication of Society material

369.33

423.75

Secretarial work for Secretary and Treasurer

59.98

Expenses of office of Secretary

12.83

Expenses of Membership Committee

37.07

Preprints of abstracts

204.40

Printing

Stamps and stamped envelopes .....		48.72
Contribution to Biologists' Smoker .....		10.00
Transferred to PHYTOPATHOLOGY for:		
Sales .....	2.00	
Subscription .....	6.50	8.50
Transferred to Lyman Memorial Fund for voluntary dues accrued to date .....		37.50
Transferred to Mycological Society for dues .....		5.00
Transferred to Classics .....		1.25
Collection charges on checks .....		2.00
Checks returned by bank .....		10.00
Total expenditures .....		\$4038.21
Balance on hand .....		5185.89
		\$9224.10

**Sinking Fund.** The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, is obtained by deducting \$5.00 from each \$10.00 life-membership installment. This fund totaled \$9466.42 at the close of 1938. During the year ending November 30, 1939, it increased to \$9571.00 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection (\$1000.00 at 6%, \$500.00 at 5%) .....	\$1500.00
Invested with following building and loan associations:	
Arlington & Fairfax Bldg. and Loan, 5% .....	1000.00
Columbia Permanent Bldg. Ass'n, 4% .....	510.00
District Bldg. and Loan Ass'n, 4% .....	1530.00
National Permanent Bldg. Ass'n, 4½% .....	1000.00
Northwestern Savings and Loan Ass'n, 4% .....	2000.00
Perpetual Bldg. Ass'n, 4% .....	1020.00
Prudential Bldg. Ass'n, 4% (interest 17.22) .....	1088.22
Less interest due PHYTOPATHOLOGY .....	\$9648.22
	77.22
	\$9571.00

The Lyman Memorial Fund, obtained from voluntary contributions, totaled \$2809.31 on November 30, 1938. During the period of slightly over a year ending December 9, 1939, this fund increased to \$3003.00, all of which is invested with the Brookland Building and Loan Association at 4%. Of the total amount \$57.56 is interest, available for PHYTOPATHOLOGY.

H. A. EDSON

**Report of the Business Manager of Phytopathology.** At the close of the year 1938 there were 638 nonmember subscriptions to PHYTOPATHOLOGY, including 5 complimentary. In 1939 there were 39 cancellations and 37 suspensions for nonpayment of dues, a loss of 76. But, with 89 new paid subscriptions, there is a net gain of 13, increasing the list at the close of 1939 to 651. Although PHYTOPATHOLOGY has a mailing list small in comparison with that of other publications, there is a definite effect upon it as a result of present world conditions, as follows: subscriptions to Czechoslovakia have dropped from 4 to 1; to China, from 23 in 1937 to 9 this year; to Japan, from 71 to 61; to Spain, from 11 in 1936 to none this year. We have already received notice of 9 cancellations to be effective for German subscriptions for 1940, more than one third of their total this year. Were it not for Soviet Russia, with a gain of 22, our list would show net decrease. The Soviet now receives 89 copies of our journal each month, compared with 188 for the United States and possessions.

#### Statement of accounts for the year ending November 27, 1939.

##### Receipts:

Balance from 1938 .....		\$3510.16
Subscriptions:		
1938 .....	\$ 116.34	
1939 .....	3303.89	
1940 .....	118.10	
1941 .....	11.35	\$3549.68

**Member subscriptions:**

1938 .....	1703.30
1939 .....	1000.00
<b>Sales of back numbers .....</b>	<b>2703.30</b>
<b>479.39</b>	

**Advertising:**

1938 .....	174.41
1939 .....	724.68
<b>899.09</b>	

**Interest on Sinking Fund:**

First-mortgage notes .....	176.57
Building and Loan .....	169.03
<b>345.60</b>	

<b>55.40</b>	
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Interest on savings account .....	70.84
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Grant from Rockefeller Institute .....	600.00
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From American Phytopathological Society for publication of Society material .....	369.33
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Allowance for reprints by printer .....	577.16
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Payment by authors for excess illustrations .....	70.99
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Reimbursement by printer .....	1.70
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First-mortgage note paid in full .....	1000.00
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<b>Total receipts .....</b>	<b>10722.48</b>
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<b>14232.64</b>
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**Expenditures:****Printing, distributing and storing PHYTOPATHOLOGY:**

Vol. XXVIII, No. 12 and Index .....	\$ 867.45
Vol. XXIX, No. 1 .....	816.90
No. 2 .....	918.98
No. 3 .....	569.87
No. 4 .....	650.23
No. 5 .....	563.68
No. 6 .....	713.91
No. 7 .....	773.09
No. 8 .....	811.19
No. 9 .....	587.63
No. 10 .....	658.83
No. 11 .....	675.77
	<b>\$8607.53</b>

Postage .....	667.74
Storage .....	48.00
	<b>\$9323.27</b>

Secretarial work and office expenses, Editor in Chief .....	372.82
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Secretarial work, Business Manager .....	222.00
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Secretarial work and office expenses, Advertising Manager .....	125.34
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Commission, Advertising Manager, 1938 .....	99.58
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Stamps and stamped envelopes .....	67.19
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Supplies .....	12.26
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Printing .....	12.34
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Reimbursement Editor in Chief for resetting type of article .....	21.52
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Refund subscription and agent's discount .....	8.45
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Reinvestment of principal .....	1000.00
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<b>Total expenditures .....</b>	<b>\$11264.77</b>
<b>Balance on hand .....</b>	<b>2967.87</b>

<b>14232.64</b>
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H. A. EDSON

**Report of Auditing Committee for the year ending November 30, 1939.** The accounts of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY are in excellent condition. We have checked statements of receipts, expenditures, bank balances, and certificates of investment, and find these correct in every detail.

December 20, 1939

E. E. CLAYTON  
ROSS W. DAVIDSON

**Report of the Advertising Manager.** Contracts for advertisements during 1939 totalled \$990.61, somewhat above the average for the past 8 years. The total number of revenue-producing advertisements was 123, occupying just under 60 pages of the journal and consisting of 21 full-page, 54 half-page, 47 quarter-page insertions, and one of one-eighth page.

There were also 45 nonrevenue-producing advertisements, occupying 38½ pages. These were made up of notices regarding Phytopathological Classics and the Society's clearing agency for employment of plant pathologists, the Directory of Advertisers, and a few complimentary and exchange advertisements.

During 1939, 17 commercial firms and one member of the Society used PHYTOPATHOLOGY as an advertising medium.

The present Advertising Manager wishes to acknowledge the help and cooperation received from Dr. Kirby in turning over the work and in securing the major part of the contracts for the year just past.

AGNES E. MEIER

**Report of the Editor in Chief.** The 29th volume of PHYTOPATHOLOGY, exclusive of the index, comprises 1077 pages of printed matter, including illustrations, and is classified as follows: One hundred seven articles, 55 notes, 4 reports of meetings, 8 book reviews, 174 abstracts (4 by title only), 247 text figures, 3 plates and 1 frontispiece. From Jan. 1 to Dec. 18, 1939, a total of 194 manuscripts of articles, notes, reports of Society meetings, and book reviews were submitted for publication in our Journal. Three of these manuscripts were withdrawn and three were rejected. Articles now in press number 25: to this should be added 122 abstracts. Additional manuscripts on hand at the time of preparation of this report numbered 47, or a total of 537 typewritten pages.

Your editor and his associates are pleased to report a continued improvement in the quality and general excellence of the manuscripts that have been and are now being submitted for publication in our Journal.

Those who experience delay in realizing publication could save themselves no little annoyance and disappointment if, before submitting their manuscripts, they would devote more and yet more attention to ordinary grammar and to concise, clear presentation of fact and interpretation. Those who contribute papers marred by such sentence construction as the following must not complain of delayed acceptance and publication:

Numerous measurements indicate that except in exposure to sun when garlic leaf temperatures are usually higher than shaded or unshaded air temperatures (though leaves in shade are at a lower temperature than shaded air) and during rain, when leaf temperatures are about equal or slightly higher than air temperatures, garlic leaf temperatures are lower than the temperature of the surrounding air.

Continued care and attention need to be given to the matter of selecting only the best and most necessary illustrations. Nothing whatever is gained by publishing illustrations that do not portray whatever of symptom, structure, or detail they are said in text or legend to show. Those contributing manuscripts containing tables should not leave to the editor the responsibility of composing suitable table headings. It sometimes happens that manuscripts carry tables wholly lacking in headings or any hint in the text as to the intent or purpose of the data presented. Your manuscript is supposed to present the final and finished distillate of your problem. Make sure that it is such before submitting it for publication. Otherwise cultivate the spirit of forbearance.

Acknowledgment is here made to Dr. F. V. Rand for his valued assistance in preparing the index of Volume 29 of our Journal and to the Science Press Printing Co. for its excellent service as printer and publisher of PHYTOPATHOLOGY.

H. B. HUMPHREY

**Report of the Manager of Phytopathological Classics for the year 1939.** I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics:

Report for the fiscal year beginning December 15, 1938, and ending December 1, 1939.		
Classics No. 1:	On hand 12-15-38	139
	Sold during year	48
	On hand 12- 1-39	91
 Classics No. 2: On hand 12-15-38		
	Sold during year	343
	On hand 12- 1-39	50
 Classics No. 3: On hand 12-15-38		
	Sold during year	443
	On hand 12- 1-39	52
		391

Classics No. 4: On hand 12-15-38	508
Sold during year	54
On hand 12- 1-39	454
Classics No. 5: On hand 12-15-38	773
Sold during year	79
On hand 12- 1-39	694
Classics No. 6: Received 2-10-39	1070
Sold	224
Given	8
On hand 12- 1-39	838
Cash balance on hand 12-15-38	\$276.50
Receipts during the year	361.28
Total	\$637.78
Expenditures:	
Postage, express, etc.	\$ 18.08
Advertising (postal cards)	16.55
Bank charges	.75
Classic No. 6: Printing	366.41
Photographs	10.50
Total expenditures	412.29
Balance on hand December 1, 1939	\$225.49
Due on accounts	\$ 10.25

H. H. WHETZEL

**Report of the Committee on Necrology.** During the calendar year 1939 there occurred five deaths of members as follows:

IVAN C. JAGGER, February 16;  
 L. M. HILL, May 6;  
 R. E. STONE, June 4;  
 E. J. PETRY, October 8; and  
 KAKUGORO NAKATA, November 14.

A. G. JOHNSON  
 M. B. WAITE

Following the reading of the necrology report, the members present stood for a moment in silence in honor of their departed colleagues.

**Report of the Committee on Biological Abstracts and the Union of American Biological Societies.** Your Committee is glad to report that during 1939 Biological Abstracts not only demonstrated that it could be maintained successfully, on the new basis put into effect in 1939, without institutional subsidy or endowment, but that it was able to provide an improved and expanded service, thanks to the cooperation of biological societies and of biologists. The plan of publishing Biological Abstracts in sections, as well as in the complete form, was appreciated and taken advantage of by many workers. Section D, *Plant Sciences*, covered plant pathology, plant physiology, ecology (with biometeorology), plant anatomy, systematic botany, agronomy, horticulture, forestry, pharmaceutic botany, pharmacognosy, paleobotany, at \$6 per year, including the complete annual indexes.

The price is not to be raised in 1940, but if 30 per cent of the members of any biological society represented in the Union, including our Society, subscribe to Biological Abstracts or to any of its sections, a reduction of \$1 will be made to each subscriber in such society, according to the plan adopted by the Trustees for the year 1940.

Among the gratifying achievements of 1939 is the doubling of the number of research journals covered systematically. In October, 1939, these numbered 1113 and no important biological journal was missed. The contributions of State and Federal research workers in plant and animal science were well covered through Government cooperation. The 18,108 abstracts published made almost an 11 per cent increase over 1938. Effective financial and editorial assistance by the Society of American Bacteriologists resulted in almost 36 per cent expansion in Section C (Microbiology, etc.). Interested groups made

possible better service in the field of biometeorology or bioclimatics, included in the Plant Science Section D. The central staff was increased from 10 to 11 with 5 temporary and 1 volunteer assistant. The number of section editors, able scientists from all biological fields who serve without pay, rose from 141 to 149. The number of copies sent out rose from 1,931 in 1938 to 2,812 in December 1939. Of the latter 1,375 were sections.

Subscriptions during this first year under the new plan brought in almost as much as the total subscription income received the year before under institutional subsidy. A balance on hand from the previous year, and other income, including sale of back volumes, society grants, and individual contributions, were sufficient to meet all 1939 expenses and leave something still in reserve. Rigid economy and efficiency in operation and the devotion of the staff contributed to make this possible. The hope is that the service may be operated permanently on the basis of income from subscriptions. The prices are set as low as possible to give as many as possible a chance to subscribe, being lower than for any similar service in its field. It is hoped that cancellations of foreign subscriptions, attributable to the war will be offset by increased domestic subscriptions.

Abstracts in 1939 appeared with gratifying promptness. For example, 82 per cent of the October abstracts were from 1939 publications. Three years before only 24 per cent in the corresponding number were from the current year. In 1939 the Annual Indexes, of a type considered unsurpassed for reference usefulness, appeared within 7 months, the shortest period on record. All previous indexes were completed and are available.

It is the hope that members of the Society and advanced students in the field will make increasing use of Biological Abstracts. Further developments of the service will depend on income made possible through additional subscriptions.

The other major activity of the Union centered about the newly launched National Association of Biology Teachers started under its auspices. Dr. Oscar Riddle, its sponsor, reported a membership approximating 2,200 at the end of 1939, and a well-established journal, *The American Biology Teacher*, appearing monthly.

HOWARD P. BARSS, Chairman

**Report of Committee on Regulatory Work and Foreign Plant Diseases.** Recognizing that all regulatory work seeking to prevent the artificial distribution of plant pests, should be based on sound, scientific, factual information, and feeling that such information is not always available, particularly in respect to regulation of foreign commerce, this committee has sought the advice and cooperation of the Division of Foreign Agricultural Service, United States Department of Agriculture.

Your President, C. R. Orton, and your Chairman, interviewed L. A. Wheeler, Chief of the Service, and received a sympathetic audience. We solicited Mr. Wheeler's cooperation in the collection, organization, and dissemination of information on foreign plant diseases. The collection of such information pertaining to causal agencies, methods of dissemination, and economic importance would be accomplished through the regular channels of the consular service; organization through the Division of Foreign Agricultural Service, and the dissemination in the United States to interested individuals through the Bureau of Plant Quarantine and Control, Plant Disease Survey, or other regular agency.

It is realized that it will take time to effectuate the objectives sought. Our present consular service and agricultural attachés are primarily interested in production records of major crops, and other purely economic matters affecting world supplies of major agricultural commodities.

Later conferences indicated that the Division of Foreign Agricultural Service has taken the first step toward educating consular officials to the need and value of information we have requested. The Bureau of Entomology and Plant Quarantine has delivered a series of lectures on the need for such information before a group of new consular appointees. It is hoped that information on phases of foreign plant diseases, essential to effective regulation of foreign commerce for the protection of American agriculture, horticulture, and forestry, will be forthcoming through the consular service in increasing annual increments, as the agricultural attachés become increasingly educated to the need.

Amendments to the Plant Quarantine Act of 1912 were proposed to Congress during the first session of the 76th Congress. A section of these proposed amendments proposed regulation of the promiscuous and unrestricted importation and interstate movement of plant pathogens, as such, in accordance with a resolution adopted by this Association at its St. Louis meeting in December, 1935. This legislation, H.R.4036 and S.1364 is still in committee in both houses of Congress.

Exempted from the provisions of the bill are "field, vegetable, and flower seeds." It is well known that plant pests are carried on seed of this character. The reasons for exemption are apparently (1) lack of information, (2) difficulties involved in port-of-entry inspection for seed-borne pathogens, although in the 1938 list of intercepted plant pests, interceptions of a number of seed-borne fungi are reported. Until more complete infor-

mation is available on world-wide geographic distribution of pathogens capable of seed distribution, and improved techniques are developed for inspecting seed importations, this country will continue to be open to invasion by foreign pathogens brought in on agricultural and horticultural seeds. The importance of this phase of regulatory work can be appreciated when it is realized that the United States imported in 1938 almost 21 thousand tons of grass and forage crop seed, and almost 13 thousand tons of garden and field seed. In the opinion of your committee the regulation of seed imports on a sound biological basis is a vital problem that we cannot afford to disregard. The subcommittee on seed-borne parasites will report progress in this matter.

Various members of the committee and others have informally discussed the possibility of international agreements pertaining to exports and imports of plant material, said agreements basically calling for restriction of export licenses to those exporters qualified for export from a pest-freedom standpoint. The importing country would reserve the right to suspend blanket permission for exports from the licensed exporters of any country if port-of-entry inspection revealed laxity on the part of the country of origin.

An examination of port-of-entry interceptions would indicate almost complete cessation of international trade in plants and plant products capable of propagation, if the importing country availed itself of the above reservation. Reciprocal arrangements between neighboring countries, or between groups of countries of similar interests and geographical situation, as proposed by H. T. Güssow, would be a gradual approach to the above system, and one offering more immediate prospect of attainment.

Because of the geographic distribution of the members of this committee, no meetings have been held. Correspondence among members has been at a minimum. This situation is not conducive to progress. It is respectfully suggested that the committee be revamped with a materially reduced personnel, in an effort to attain a functional committee organization.

It is further recommended that the committee be instructed to investigate the foreign pest records of the major species of plants or seeds imported annually, and report at our next annual meeting.

Respectfully submitted,

RICHARD P. WHITE, Chairman, H. T. GÜSSOW, J. S. BOYCE, W. A. McCUBBIN,  
R. D. RANDS, J. F. ADAMS, E. L. CHAMBERS, AND M. T. MUNN

**Report of Extension Work and Relations Committee.** The Extension Work and Relations Committee sponsored two activities in 1939: The first was an evening conference on tobacco diseases, held in Greenville, Tennessee, August 9, the occasion being the annual meeting of the Tobacco Disease Council. This meeting was attended by extension plant pathologists from Virginia, Georgia, North Carolina, Ohio, and the United States Department of Agriculture. Most of the discussion was centered around control treatments for tobacco downy mildew.

The second activity was a conference on the subject: "Recent Studies on Fire Blight of Apples and Pears," which was held on the afternoon of December 28, at the Columbus Meeting. The discussion was centered around the epidemiology and control of fire blight. The principal speakers on the program were G. W. Keitt and E. M. Hildebrand. A detailed report of this conference will appear in the Extension Plant Pathologist in the near future. The attendance at this conference was about 75.

LUTHER SHAW, Chairman, CHAS. CHUFF, R. J. HASKELL, A. L. PIERSTORFF,  
R. S. KIRBY, E. C. STAKMAN, G. W. KEITT, W. B. TISDALE, J. L. CONNERS

**Report of the Committee on Coordination in Cereal and Vegetable Seed Treatment Research, 1939.** Experiments were made to determine the effectiveness of certain fungicides in controlling smuts and seedling diseases of wheat, oats, and barley, and to determine the value of some of these fungicides in increasing yields of these crops. These experiments were carried on at 10 stations in the northern United States, and 3 stations in Canada. Seed lots were selected, treated, and packaged at University Farm, St. Paul, Minnesota, and sent to the cooperating stations. At the present time, complete and excellently prepared reports have been received from 6 of these stations.

The experiments were divided into 2 separate groups, i.e., disease-control tests, and yield tests. In the disease-control tests, seed of artificially smutted, susceptible varieties of wheat and oats and of naturally stripe-infected barley was treated with 5 or 7 different fungicides and was planted in 6- or 8-foot rows replicated 3 times. Notes were taken on the development of disease and on seedling stand.

In the yield tests, fewer fungicides were used on commercial varieties of wheat, oats, and barley. The seed was not artificially inoculated but was moderately infected with common seed-inhabiting fungi. Only yield notes were taken on these tests, which were planted in rod rows replicated either 5 or 10 times. Notes on various ecological factors were recorded for both sets of experiments.

Experimental results have not yet been analyzed statistically, but the data indicate that New Improved Ceresan and DuBay 1155-IW are the most effective chemicals for the control of smuts and barley stripe. Leytosan is less effective for the control of barley stripe and is not at all satisfactory for the control of oat smuts. Formaldehyde dusts, which are generally less effective against oats smuts than New Improved Ceresan, under certain conditions, may be more effective. For example, at Wyoming, where 64 per cent of smut developed in the check, there was only 12 per cent smut in the formaldehyde-dust treatments as compared with 23 per cent in the New Improved Ceresan treatment.

Summaries of all of the data will be sent to each of the collaborators as soon as all of the reports have come in and have been analyzed statistically. No conclusions can be drawn from the yield tests without the aid of statistical analyses.

J. G. Horsfall has prepared a summary of "State Recommendations on Vegetable and Flower Seed Treatment," which he has offered to mimeograph and distribute to the various States. From this report it is evident that a wide disparity exists between the recommendations of the different stations.

No experimental work has been undertaken with corn, flax, or any of the vegetables, and no definite plans have been made for the future, but it is recommended that the present experiments be continued. The committee welcomes criticisms on the present work and invites suggestions for experiments with corn, vegetables, and other crops.

M. B. MOORE, Chairman, W. E. BRENTZEL, F. J. GREANEY,  
J. G. HORSFALL, H. A. RODENHISER

**Report of the Committee on Standardization of Fungicidal Tests.** The work of the Committee has been divided into 3 phases, with specially designated subcommittees as follows: (A) Laboratory Methods—S. E. A. McCallan and J. G. Horsfall, in cooperation with F. Wilcoxon and J. W. Heuberger; (B) Field Methods—H. C. Young and K. J. Kadow; and (C) Laws regulating the sale of fungicides—J. W. Roberts, in cooperation with Errett Wallace.

**Laboratory and Field Methods.** In the furtherance of standardization of laboratory and field methods it is proposed to develop "Standard Methods" as follows: A method having been studied by several different laboratories and found satisfactory will be presented as a "Tentative Method," mimeographed and distributed to members of the Society and others interested. After the method has been adequately tested and reported back to the committee, it will be either (1) adopted as a Standard Method, (2) modified for further testing, or (3) discarded. A Standard Method would be published under the authority of The American Phytopathological Society, Committee on the Standardization of Fungicidal Tests. In the event of studying potential, tentative methods, it becomes necessary to perform original research, such results would become the property of the investigators, to be published as they saw fit. A tentative or Standard Method would not in itself constitute original research, but would cite all previous and original contributions.

The following Tentative Methods have been proposed:

1. Tentative method on a standard Bordeaux mixture for laboratory tests and for determination of Bordeaux coefficient.
2. Tentative method for determination of mean particle diameter of fungicides.
3. Tentative specifications for slide-moist-chamber method of testing protective fungicides.
4. Tentative recommendations on standard spray nomenclature.

Members of the Society and others interested are invited to cooperate by testing and criticizing these tentative methods. Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

Continued cooperation in developing laboratory and field methods, and in correlation of laboratory and field results are in progress. A cooperative study of methods of evaluating apple foliage injury, embodying original research, was begun and will be continued next season.

A round-table conference on laboratory testing of fungicides was sponsored by the Committee at the Columbus meeting. A large and responsive group attended. Informal reports were given on Standardization of the fungus, factors involved in deposition of sprays, evaluation of results, indications on predicting field performance, and difficulties and inconsistencies of laboratory tests.

**Laws Regulating the Sale of Fungicides.** The subcommittee, having studied this question, points out the desirability of uniform State and Federal laws governing the sale of fungicides. Manufacturer, wholesaler, retailer, user, and experimenter would be benefited, and regulatory work would be more effective and less expensive. The cooperation of various interested groups to formulate uniform regulations would be desirable. It is further suggested that the service of the plant pathologist to the user of fungicides would be greatly facilitated if on every package of fungicidal material there was (1) a complete statement of composition and (2) a statement of accurate directions for use.

It is recommended that the Society go on record as favoring these suggestions.

It is proposed that the subcommittee continue its investigations by cooperating with various interested groups such as the entomologists, horticulturists, and manufacturers in an attempt to arrive at a mutual basis for formulating uniform regulations.

S. E. A. McCALLAN, Chairman, J. W. ROBERTS, H. C. YOUNG,  
K. J. KADOW, J. G. HORSFALL, C. E. YARWOOD, R. H. DAINES

**Report of the Advisory Committee for 1939.** The advisory committee on programs and society activities has given attention during the year to numerous suggestions submitted to it by the membership. We have endeavored to sound out society opinion in a number of representative centers on the character of any changes that might be advantageously suggested to the program committee. The most impressive result of this survey is the general satisfaction with our programs and the comparative lack of general criticism.

There is fairly uniform agreement on certain matters, which have been suggested to the council as follows:

(1) The presentation of new material in the form of exhibits might well receive greater emphasis.

(2) The policy of encouraging groups to get together to discuss problems of common interest in special programs or in informal gatherings is appreciated generally. It, of course, goes without saying that this should not expand to the point of jeopardizing the unity of the general program.

(3) While there is not unanimous agreement on the question as to whether or not a policy of enlarging upon the number of invitation papers should be adopted, the majority opinion is decidedly in the negative.

(4) A majority seem to feel that the length of our session is not excessive. There are many of the opinion that too many papers are accepted, although it would appear that this group is still in the minority. The continuance of the democratic spirit that has prevailed in the acceptance of papers from members is very definitely desired. Any increased authority in the hands of a small committee empowered to select or reject papers on their merits apparently would not be approved by the majority. If it becomes necessary in future to restrict the number of papers at a session to a maximum number, it is suggested that this be accomplished by a general rule of limitation, such as the restriction of a number of papers per member.

J. C. WALKER, Chairman, F. L. DRAYTON, A. A. DUNLAP, M. W. GARDNER,  
E. L. NIXON, R. K. VOORHEES, W. J. ZAUDEMAYER

**Report of the Membership Committee.** This committee has continued its effort to increase the membership in The American Phytopathological Society. A high-pressure type of campaign has been studiously avoided. Whatever progress has been made we owe to the active cooperation of society officers and a large number of individual society members.

The efforts of the committee have been directed along the following several lines:

1. A circular letter has been sent to each member.

2. A representative in each State and Canadian Province has been asked to canvass the local situation and invite suitable former members and nonmembers to join. The local list of members has been sent to each one.

3. Letters have been written to all former members who have been dropped during the last several years because of nonpayment of dues.

4. Letters have been sent to each promising nonmember scientist who has had a paper cited in the current volume of *PHYTOPATHOLOGY*.

5. Sixty-two new members were elected and 14 former members were reinstated in 1939.

A. J. RIKER, Chairman, J. C. CARTER, KENNETH KADOW,  
R. S. KIRBY, R. M. LINDGREN, B. A. RUDOLPH

**Report of the Committee on Technical Words.** In view of the fact that the International Botanical Congress, scheduled to convene in Stockholm, Sweden, in 1940, has been postponed, your committee on technical words feels justified in submitting two separate and not wholly consistent lists of terms, with the request that these be printed as a part of this report in order that they may be generally available to the members of the Society, and in suggesting that formal action by the Society be deferred until all members have opportunity to appraise the definitions submitted. One list was recently submitted by G. Wilbrink and contains definitions in French, German, and English. The other list is made up of definitions on which the members of this committee are more or less tentatively agreed. In preparing these definitions we have tried to follow the most common and established usage, were it precise enough, otherwise what seemed to be the most correct usage was followed, consideration being given to usage in other countries. We also have tried to make each definition a unit; that is, understandable without references to another definition, unless it referred to one immediately preceding.

To these lists we venture to prefix some observations regarding the value, as well as the intrinsic difficulty, of the interesting task we somewhat rashly undertook. We are encouraged in this procedure by the earlier action of the Committee on Nomenclature of the Ecological Society (see *Ecology* 20: 331-333. 1939) whose "basic principles" will repay a careful reading. Anyone really interested in principles of definition will find a number of other relevant references in the text of the following paragraphs.

Dr. Wilbrink and the members of this committee will, of course, welcome suggestions, corrections, and additions. Indeed, if criticisms are not forthcoming, the chief purpose of publication will have been missed. It may be well to emphasize in advance, however, that while we do not regard our definitions as final, or indeed, in some cases, as very satisfactory, we believe that even an imperfect definition may be well worth study.

Considering the number and variety of papers published, language should necessarily serve as an aid instead of a hindrance to understanding, that the reader should not have to search for the author's meaning, depend too much on context, or be obliged to "translate" into common usage. Inevitably, the meaning of a word depends in the long run on usage, but definition is only an aspect of usage. A definition is implicit in every use of a word. Explicit definition records and tends to limit usage.

It should perhaps be repeated that we are under no delusion as to the possibility of inducing uniformity of usage. We feel, however, that something may be done to aid in reducing loose use of technical words. Loose usage is not the same as different usage. An author may use words in a sense quite different from the usual, but, if his concepts are clearly explained and his meanings explicitly defined, consistent with each other and consistently applied by him, he cannot be accused of loose usage. Different usage, however, in itself offers special dangers, particularly if it involves coined or little-known words. One of the extreme examples of this in English botanical writing is the subject of comment by L. A. Walford and G. S. Myers in the current number of *Copeia*, December 26, 1939, page 240.

The whole purpose of language is to convey ideas to other persons. If each person or group used words in a different way, there would be hopeless confusion of meanings. To a lesser degree the same thing happens if a word or a group of words be used in a manner different from the usual. A certain amount of conformity to accepted usage is necessary to understanding. Definitions should be an aid to attaining the necessary minimum of uniformity.

It is obvious that there is a wide difference in the need for exactness in definition and use. The loose use of such terms as *disease* and *epidemic* causes little confusion or misunderstanding, whereas continued misuse of such words as *immunity*, *resistance*, *tolerance* and *kleindusity* (with *resistance* as a catch-all) tends definitely to confuse or mislead readers. The difficulty is, of course, enhanced when the attempt is made to translate such loosely used terms.

As an illustration of the intrinsic difficulty of defining the sort of terms in which we are most interested, take the two definitions of the word *parasite* in the attached lists. One includes and the other excludes the viruses. There is already very good authority for the use of *host* and *parasite* in connection with viruses. Whatever viruses may finally prove to be, they are certainly, as a group, dependent for their existence on the organisms they infect, as absolutely dependent as such obligate parasites as the rust fungi. They are known to multiply or be propagated, and must in some way obtain material for this spread from an organism which would, therefore, function as host for the virus. Yet so to define the word *parasite* as to include them apparently involves either an extension of the term or the assumption that viruses are *living* things.

In an essay on "The meaninglessness of the terms life and living," recently published by the Cambridge University Press in a volume entitled "Perspectives in Biochemistry," N. W. Pirie points out that in scientific nomenclature there is a whole class of ordinary English words whose meaning the scientist rather gratuitously redefines. *Life* and *living* are clearly words that the scientist has borrowed. The loan has worked satisfactorily until comparatively recently, for the scientist seldom cared and certainly never knew just what he meant by those words. Pirie finally arrives at the conclusion that "until a valid definition has been framed it seems prudent to avoid the use of the word 'life' in any discussion about border-line systems."

While one may always temporarily solve a problem by giving it up, and it is possible, though not convenient, to avoid a term such as *life*, there are terms that we need to use, even though we know our concepts may soon change and that any definition probably will soon be modified. Thus, it seems desirable to attempt a definition of *virus* in spite of the general uncertainty as to the nature of viruses.

In P. W. Bridgman's "The Intelligent Individual and Society" (1939) words and their uses are discussed in a number of places. For example, on pages 18, 19, and 24, which lead up to the statement on page 56 that "The difficulty [in the use of words]

<sup>1</sup> By permission of The Macmillan Company, publishers in U. S. A.

is enhanced by the fact that words in language are part of a stream of activity. The meaning of a word is affected to some extent by the whole context in which it is embedded, and, since this context never exactly recurs, the meaning of the word is never precisely the same." More to the point right here is a specific reference to the question, "What is life?" found on page 78. It follows a discussion of "footless" questions and points out that many questions which on their face have some "objective" significance, in reality are concerned with verbalisms. "For instance, the question 'What is life?' turns out on analysis not to be a question about the external world alone, but a question as to the adequacy of our classification of the objects of the external world into living and dead."

In order to convince oneself that the dilemma at which we have arrived is by no means confined to plant pathology, it is only necessary to read any one of a number of recent scientific books. For example, in the introductory chapter to "The Human Value of Biology," published in 1938 by the Harvard University Press, Johan Hjort quotes and paraphrases Kant to the effect that whereas an arbitrary concept can always be defined, it must be very difficult and perhaps impossible, to define objects in nature. "Consequently the science of mathematics alone possesses definitions."

In spite of all these admitted obstacles, the members of your committee, again urge the publication, as a part of this report, of the lists submitted herewith, and of their continued revision by subsequent committees, lest the Society fall under the condemnation expressed by Lamarck in relation to a quite different subject. (See The Lamarck Manuscripts at Harvard, edited by Wheeler and Barber, page 161). "The majority of men consider only the words they employ without disturbing themselves seriously about the ideas they intend to express. Everyone interprets words to suit himself according to his lights, his tastes and his desires. . . ."

JESSIE I. WOOD  
NEIL E. STEVENS  
DONALD REDDICK, Chairman

#### DEFINITIONS SUBMITTED BY THE COMMITTEE ON TECHNICAL WORDS

**ATTENUATION:** Lessening of the capacity of a parasitic organism or virus to cause disease in the host; reduction in its virulence.

For the opposite process, *restoration* has been used but is not complete in itself, i.e., must be used in the expression *restoration of virulence*. Other words suggested are *reversion*, *revigoration*, *revirulence*.

**CARRIER:** An individual invaded by a pathogenic organism or virus without obvious reaction or sign of injury.

**DISEASE:** Deviation from normal functioning of physiological processes, of sufficient duration or intensity to cause disturbance or cessation of vital activity.

Difficulty or failure in the vital processes of an organism. (Adapted from Link, p. 847.)

**HOST:** Living organism harboring another organism or virus dependent on it for existence.

**HYPERSensitivity:** Violent reaction of an organism to attack by a pathogenic organism or virus, with prompt death of invaded tissue preventing further spread of infection.

(Hypersensitiveness.) (Necrogenetic abortion in Wilbrink's list.)

(Intolerance as used for some virus diseases is apparently the same phenomenon.)

**IMMUNITY:** Freedom from disease, due to lack of qualities permitting or to possession or acquirement of qualities preventing the operation of the pathogenic factor.

With special reference to parasitic diseases: Freedom from attack by a pathogenic organism or virus due to lack of qualities corresponding to its requirements or to possession or acquirement of additional qualities unfavorable to it.

**NATURAL IMMUNITY:** Immunity due to qualities inherent in an individual.

**ACQUIRED IMMUNITY:** Immunity acquired during the lifetime of the individual organism (not certainly demonstrated to occur in plants, with the possible exception of certain virus diseases).

**IMMUNIZATION:** Treatment of an organism designed to render it exempt from attack by a given pathogenic organism or virus; process of acquiring immunity.

**IMMUNE:** Exempt from disease; not subject to attack by a pathogenic organism or virus. With *from*; also used (erroneously) with *to*; (and, according to Webster's Dictionary, with *against* or *of*). *From* is generally considered to be best usage.

<sup>2</sup> Reprinted by permission of the President and Fellows of Harvard College.

**INFECT:** (Of a pathogenic microorganism or virus)—to invade an organism.

(Of an agent)—to affect an organism with a pathogenic microorganism or virus; to bring about infection in an organism.

**INFECTED:** (Of an organism)—Invaded by a pathogenic microorganism or virus.

Following most precise usage it is preferable to restrict the application of the term *infected* to an organism actually invaded by a pathogenic microorganism or virus. For mere surface contact or mixture with microorganisms, as of spores on seeds, etc., or for inorganic substrata, it seems best to use the term *contaminated*, as contaminated seed, or soil, etc., although many careful writers do use *infected*. The term *infested* is preferred by some to *contaminated* for use in this connection, whereas others following what appears to be the best non-technical English usage confine the application of *infested* to organic or inorganic substrata invaded by larger foreign organisms.

**INFECTIBLE:** Possessing qualities permitting invasion by a pathogenic microorganism or virus.

**INFECTIBILITY:** noun.

**INFECTION:** Process or state of establishment of a pathogenic microorganism or virus in a living organism; state produced in the affected organism by such establishment.

**INFECTIOUS:** Resulting from invasion by a pathogenic microorganism or virus (of disease); communicable; transmissible.

**INFECTIVE:** Possessing ability to produce infection; productive of infection.

(Of a microorganism or virus)—possessing ability to invade a living organism.

(Of a vector, medium, etc.)—possessing ability to transmit a pathogenic microorganism or virus.

**INFECTIVITY** (infectiveness) : noun.

**INOCULATE:** To contaminate or mix, to bring into contact or implant (an organism, culture medium, soil, etc.), with, a microorganism or virus, or material containing either. Used with *with*.

To introduce a microorganism or virus, or material containing either, into (an organism, culture medium, soil, etc.). Used with *into*, sometimes with *to*.

It does not seem worth while to discourage the common and well-established usage with reference to inorganic substrata, often criticized on the grounds that one cannot inoculate that which cannot become diseased.

**INOCULATION:** noun.

*Of* (a medium, organic or inorganic) *with* (a microorganism or virus).

*Of* (a microorganism, etc.) *into* (a medium).

*By* (an agent or method).

**INOCULUM:** Material acting or used in natural or artificial contamination with a microorganism or virus.

**INOCULA:** plural.

**KLENDUSITY:** Ability of a susceptible variety to escape infection because of possession of some quality preventing or hindering successful inoculation under conditions conducive to infection in other varieties.

**PARASITE:** Organism or virus existing within or attached to or in intimate association with another living organism, from the functioning tissues of which it derives part or all of the material for its nutrition.

Organism or virus for which the tissues of another living organism serve as substratum and source of nutrition.

**PAREASITISM:** Partial or complete nutritional dependence of one organism or virus on the tissues of another living individual.

The above definitions do not exclude such structures as embryo sacs and the sporophytes of Bryophyta. If this is desired it will be necessary to qualify the phrase *other living organism* by some such phrase as *in whose development or functioning it is not a necessary or normal part*.

**PATHOGEN:** Parasitic organism or virus whose activity causes disease in the host.

**PATHOGENIC:** Disease-inciting; possessing ability to induce disease.

**PATHOGENICITY:** Ability to cause disease.

**RESISTANCE:** Ability of an organism to withstand or oppose the operation or to lessen or overcome the effects of an injurious or pathogenic factor.

Ability of the host to suppress or retard the activity of a pathogenic organism or virus.

**SENSITIVITY:** Inability of the affected organism to endure the operation of an injurious or pathogenic factor or the activity of a pathogenic organism or virus without more or less strong reaction, evidenced by varying degrees of symptom expression and damage.

(Sensitivity.) (Sensibility, in Wilbrink's list.)

**SUSCEPT:** Organism affected or capable of being affected by a given disease.

**SUSCEPTIBILITY:** Inability of an organism to oppose the operation or to overcome the effects of an injurious or pathogenic factor.

Inability of the host to defend itself against or to overcome the effects of invasion by a pathogenic organism or virus.

**TOLERANCE:** Ability of the affected organism to endure the operation of a pathogenic factor or invasion by a pathogenic organism or virus with little or no reaction, as shown by the more or less complete absence of symptom expression and damage.

(In Wilbrink's list: Nonsensitivity plus tolerance.)

**VIRULENCE:** Relative capacity to cause disease; degree or measure of pathogenicity of a parasitic organism or virus.

**VIRULENT:** Manifesting a high degree of pathogenicity; strongly pathogenic.

**AVIRULENT:** opposite of virulent.

**VIRUS:** An obligately parasitic pathogen, capable of reproduction in suitable hosts, *ultra-microscopic* and recognizable only because of the visible effects produced in the infected host. (Derived from Bawden's text. See below.)

"... an obligately parasitic pathogen with at least one dimension of less than 200 m.p." (as defined by F. C. Bawden in "Plant Viruses and Virus Diseases" published by the Chronica Botanica Company, Leiden, Holland. 1939.—Further, discussing the possibility of the existence of saprophytic viruses or parasitic viruses that cause no symptoms in any infected organisms, "... until such saprophytes or hypothetical parasites can be shown to resemble the pathogenic viruses in properties more fundamental than size, it would seem preferable to restrict the name virus to agents fulfilling all three requirements of the suggested definition.")

"Probably the majority of scientists at the present time are of the opinion that viruses are high molecular weight proteins capable of reproduction in a specialized medium, that medium perhaps being restricted to living protoplasm" of infected organisms in which they cause disease. (C. W. Bennett, The nomenclature of plant viruses. *Phytopath.* 29: 422-430. 1939.)

The established plural form in English is VIRUSES.

According to Holmes (Francis O. Holmes, Proposal for extension of the binomial system of nomenclature to include viruses. *Phytopath.* 29: 431-436. May 1939) the Latin word *virus* was not used in the plural, but if a plural were formed it would be *vira*. He has used this coined Latin plural form as the name of a suggested new organic kingdom, *Vira* (*l.c.*).

**VIROSIS:** A disease caused by a virus, virus disease.

The use of *virosis*, *viroses*, has been objected to because of suggested possibility of confusion with *viruses*. This possibility seems remote and insufficient grounds for the rejection of a useful and established word.

#### DEFINITIONS SUBMITTED BY DR. G. WILBBINK

**PARASITE:** Organism capable of growing and (or) multiplying on or within a living plant, the host, and of deriving part or all of its food from the functioning tissues of this plant, a one-sided nutritive relationship never beneficial and often harmful to the host.

**AGGRESSIVITY OR VIRULENCE:** Ability to live as a parasite.

**PATHOGEN:** Parasite, virus or other agent capable of inducing or inciting disease.

**PATHOGENICITY:** Ability to induce or incite disease.

**ATTENUATION:** Lessening of pathogenicity.

**RESTORATION:** Restoration of pathogenicity.

**SUSCEPTIBILITY:** The sum total of qualities which make a plant a fit host for a given pathogen.

**NONSUSCEPTIBILITY:** The lack of the above qualities.

**RESISTANCE:** The sum total of qualities of the host which oppose the development of a given pathogen.

**NATURAL PASSIVE OR STATIC RESISTANCE:** Resistance which is due to qualities innate to the host prior to the attack and not to reactions incited by the attack.

**NATURAL ACTIVE OR DYNAMIC RESISTANCE:** Resistance which is due to reactions incited by the attack.

**IMMUNIZATION:** Treatment applied to a plant in order to increase its resistance or to confer resistance upon it.

**INDUCED RESISTANCE:** Resistance or increase of resistance if the plant reacts passively in regard to this treatment.

**ACQUIRED RESISTANCE:** Resistance if the plant reacts actively in regard to this treatment.

**SENSITIVITY:** Liability of the plant to show more or less violent symptoms of disease.

**NONSENSITIVITY:** Ability of the plant to endure the development of a parasite or a virus or the influence of another pathogenic agent without showing symptoms of disease.

**TOLERANCE:** Ability of a plant to endure the development of a parasite or a virus or the influence of another pathogenic agent without showing more than slight symptoms of disease.

**ABORTOGENIC NECROSIS:** The prompt death of the host tissue at the point of attack of the pathogen, checking the further development of the latter.

#### NOMENKLATUR PHYTOPATHOLOGIE

**PARASIT:** Organismus, der die Fähigkeit besitzt in oder auf einer lebenden Pflanze (Nährpflanze oder Wirt) zu leben oder (und) zu multiplizieren und dabei seine Nahrung teilweise oder ganz den funktionierenden Geweben dieser Pflanze zu entnehen, welche einseitige Nahrungsgemeinschaft nicht vorteilhaft sondern oft schädlich ist für die Nährpflanze.

**AGRESSIVITÄT ODER VIRULENZ:** Die Fähigkeit um parasitisch zu leben.

**PATHOGEN:** Parasit, Virus oder sonstige Erreger im Stande Krankheitsscheinungen bei Pflanzen zu erregen.

**PATHOGENITÄT:** Die Fähigkeit Krankheitsscheinungen bei Pflanzen zu erregen.

**ABSCHWÄCHUNG:** Verringerung der Pathogenität.

**WIEDERHERSTELLUNG:** Restauration der Pathogenität.

**ANFÄLLIGKEIT ODER EMPFÄNGLICHKEIT:** Die Summe der Eigenschaften, die eine Pflanze geeignet machen als Nährpflanze für einen Parasit oder einen Virus zu dienen.

**UNANFÄLLIGKEIT ODER UNEMPFÄNGLICHKEIT:** Der Mangel an den genannten Eigenschaften.

**RESISTENZ ODER WIDERSTANDSFÄHIGKEIT:** Die Summe der Eigenschaften der Nährpflanze, welche die Entwicklung oder (und) Vermehrung des Parasiten oder Virus hemmen.

**NATÜRLICHE PASSIVE ODER STATISCHE RESISTENZ:** Die Resistenz, welche hervor aus Eigenschaften geht, die schon vor dem Angriff des Parasiten oder Virus anwesend waren und nicht aus Reaktionen durch den Angriff erregt.

**NATÜRLICHE AKTIVE ODER DYNAMISCHE RESISTENZ:** Die Resistenz, welche auf Reaktionen beruht, durch den Angriff erregt.

**IMMUNISIERUNG:** Behandlung einer Pflanze mit dem Zwecke die Resistenz dieser Pflanze zu erhöhen oder ihr Resistenz zu verleihen.

**INDUZIERTE RESISTENZ:** Die Resistenz oder Erhöhung der Resistenz, wenn die Pflanze sich bezüglich dieser Behandlung passiv verhält.

**ERWORBENE RESISTENZ:** Die Resistenz, wenn die Pflanze sich bezüglich dieser Behandlung aktiv verhält.

**EMPFINDLICHKEIT:** Beschaffenheit der Pflanze um mehr oder wenig starke Krankheiterscheinungen zu zeigen.

**UNEMPFINDLICHKEIT:** Vermögen der Pflanze die Entwicklung oder (und) Vermehrung eines Parasiten oder Virus oder die Einwirkung eines anderen pathogenen Einflusses zu ertragen ohne Krankheiterscheinungen zu zeigen.

**TOLERANZ:** Vermögen der Pflanze die Entwicklung oder (und) die Vermehrung eines Parasiten oder Virus oder die Einwirkung eines anderen pathogenen Einflusses zu ertragen und keine oder nur undeutliche Krankheiterscheinungen zu zeigen.

**ABORTOGENE NEKROSE:** Das schnelle Absterben des Gewebes der Nährpflanze an der Angriffsstelle des Parasiten oder Virus, wodurch der Entwicklung des Parasiten oder Virus ein baldiges Ende gesetzt wird.

#### NOMENCLATURE PHYTOPATHOLOGIE

**PARASITE:** Organisme étant capable de se développer ou (et) de se multiplier sur ou dans une plante vivante (plante nourricière ou sujet) et d'extraire une part de sa nourriture ou toute sa nourriture des tissus fonctionnantes de cette plante, cette association nutritive uni-latérale n'étant pas avantageuse mais souvent nuisible au sujet.

**AGRESSIVITÉ OU VIRULENCE:** La capacité de se développer ou (et) de se multiplier dans une plante vivante.

**PATHOGÈNE:** Parasite, virus ou autre cause capable d'engendrer ou d'inciter des symptômes de maladie végétale.

**PATHOGÉNICITÉ:** Capacité pathogène.

**ATTÉNUATION:** Affaiblissement de la pathogénicité.

**BESTAURATION:** Restauration de la pathogénicité.

**RÉCEPTIVITÉ:** Le total des qualités, qui rendent une plante accessible à un parasite ou un virus.

**RÉSISTANCE:** Le total des qualités du sujet, qui s'opposent au développement ou (et) à la multiplication du parasite ou virus.

**RÉSISTANCE NATURELLE PASSIVE OU RÉSISTANCE STATIQUE** est basée sur des qualités déjà présentes dans le sujet antérieurement à l'attaque du parasite ou du virus et non produites par l'attaque.

**RÉSISTANCE NATURELLE ACTIVE OU RÉSISTANCE DYNAMIQUE** est basée sur des réactions produites par l'attaque.

**IMMUNISATION:** Traitement d'une plante afin d'en augmenter la résistance ou de lui conférer de la résistance.

**INDUITE** est cette augmentation ou obtention de résistance d'une plante passive en rapport du traitement.

**ACQUISE** est cette augmentation ou obtention de résistance d'une plante active en rapport du traitement.

**SENSIBILITÉ:** Aptitude d'une plante de montrer des symptômes de maladie.

**INSENSIBILITÉ:** Capacité d'une plante de subir le développement ou (et) la multiplication d'un parasite ou virus ou l'action d'une autre cause pathogène sans montrer des symptômes de maladie.

**TOLÉRANCE:** Capacité d'une plante de subir le développement ou (et) la multiplication d'un parasite ou virus ou l'action d'une autre cause pathogène sans faire paraître des symptômes évidents de maladie.

**NÉCROSE AVORTOGÈNE:** La mort subite du tissu au point d'attaque du parasite ou virus arrêtant la marche du pathogène.

**Report of the Temporary Committee on Fungous Nomenclature.** In the past summer the President of the Society appointed a committee on plant pathology for the purpose of cooperating with a similar committee of the British Mycological Society. The purpose involved was twofold: first, to work toward a stabilized nomenclature for the fungi, particularly for those species involved as plant pathogens, and secondly, to consider again after the lapse of some years the possibility of the standardization of common names of plant diseases. The British committee has made notable progress along both lines through concrete proposals for *nomina generica conservanda* (see *Trans. Brit. Myc. Soc.* 23: 215-232, 281-292, 1939) and in the publication of a list of common names of British plant diseases. This list is now in its second edition, and a third has been in preparation. Both the common names employed in this list and the corresponding Latin names of the pathogens have had wide acceptance throughout the British Empire.

The American committee is giving careful thought to both phases of the problem. As a preliminary step, the proposal of the British committee for the conservation of *Urocystis* versus *Tuburcinia* will be seconded by a note in *PHYTOPATHOLOGY*. Further consideration will be given to other genera for conservation in accordance with the International Rules of Botanical Nomenclature, and it is hoped a definite beginning can be made on the admittedly difficult task of arriving at a generally acceptable series of common names for American plant diseases.

G. L. ZUNDEL, Chairman, J. A. STEVENSON,  
C. M. TUCKER, D. S. WELCH, ERDMAN WEST

**Report of Temporary Committee on Publicity.** Your temporary committee on publicity wishes to report that it finds immense possibilities of popularizing phytopathology and improving its public relations. To accomplish this the committee should function throughout the year. It is only by the sincere and unselfish cooperation of the members that these relations can be suitably maintained.

The committee can act only as a clearing house of information furnished by the pathologists of the country through the committee to the science writers, reporters, and magazines. It is not the purpose of the committee to replace any local publicity, but rather to further national distribution of new information and correlate this information with plant pathologists, giving full credit to the author.

We suggest that the committee be made permanent.

C. T. GREGORY, Chairman, J. A. PINCKARD, A. J. ULLSTRUP,  
J. H. JENSEN, H. W. RANKIN, W. S. SNYDER

**Proposed Policies and Functions of the 1940 Standing Committee on Publicity and Public Relations.** It is the sentiment of the Society that plant-pathological problems are not receiving their proper recognition in the press and that this lack of publicity is seriously affecting the recognition and support of our work. A committee of 10 members was appointed to study this problem and to improve our publicity relations.

It is not the function of this committee to interfere in any way with the local or national publicity arrangements that may already be extant, but rather to serve as a tool to further implement our publicity. Its success depends solely on the whole-hearted cooperation of the pathologists of the country.

The functions and policies of the committee should be:

1. To serve as a contact agent between pathologists and the various news agencies to the end that plant pathological news items may be given wide publicity.
2. To create and maintain public interest in plant diseases and their control by showing that crop losses caused by these diseases may affect both farm and city people and by keeping the people informed of the latest developments in control.
3. To give full credit to the investigator or source of the information.
4. To release such publicity immediately after it has been published in a scientific journal, unless specifically permitted by the author to release the information immediately upon its receipt.
5. To issue dignified, factual news reports to accredited science-news writers and to suppress inaccuracies and sensationalism through indiscriminate releases to unknown and

untried science writers. It is not the committee's intention to release news items to local papers but to the national chains that employ the very best science writers.

C. T. GREGORY, Chairman, O. C. BOYD, J. H. JENSEN, J. A. PINCKARD, G. H. STARR,  
FRANK MCWHORTER, A. G. NEWHALL, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG

**Summary of Report of Committee on Proposed Cumulated Index for PHYTOPATHOLOGY.** It is recommended that the Council of The American Phytopathological Society authorize the preparation and publication of a cumulated index covering the first 30 volumes of *PHYTOPATHOLOGY*, and it is suggested that the program be financed by funds to be raised by the Committee on Donations and Legacies, with eventual reimbursement, at least in part, from receipts on the sale of the Index, unless a better plan be forthcoming.

A statistical study of the first 28 volumes of *PHYTOPATHOLOGY* indicated an average of 20 index pages per volume, with 9 titles covered per index page. This would work out to 600 pages for the 30 volumes. However, due to much combining of entries in a cumulated index, it is estimated that the 30-volume index would not run over 400 pages, and very probably it would be much less, using the general type of indexing employed in the current volume.

It is recommended that qualified pathologists be invited to index one volume each at an honorarium of \$25.00 for 12 issues (2 volumes for the earlier years, and 1 volume beginning with 1918), the index entries to be typed on perforated sheets to be furnished them, along with directions for indexing.

It is estimated that the total cost of preparing the index for the printer would not exceed \$1,000.00, including honoraria, correspondence, index paper, and clerical work (alphabetizing and typing). The printer's bill, depending on the paper stock used and the number of pages, should not exceed \$2,100.00 to \$2,400.00 (including mail). An edition of 1,500 is suggested. A tentative quotation as to price for pre- and post-publication orders, respectively, might be \$3.50 and \$3.75 to members, and \$3.75 and \$4.00 to non-members, but perhaps higher figures and a wider spread in price would be more desirable.

If approved, (1) work should go forward immediately, and (2) an announcement should appear in *PHYTOPATHOLOGY*. The Committee is not in complete agreement as to the advisability of circularizing the membership at this stage for pledges to purchase the Index when published. Which step should precede is probably a matter for the Council to decide.

The full report of the Committee follows:

*Report of Committee on Proposed Cumulated Index for Phytopathology.* In the spring of 1939, preliminary to any discussions or actions by this Committee, a study was made of the 28 volumes of *PHYTOPATHOLOGY* then completed, counting the pages of text and of index, numbers of papers, abstracts, reviews, and notes, the total number of titles (sum of last four items) indexed, number of titles per index page, and average number of text pages per title for each of these volumes. These data are summarized in the tabulation on page 370.

Miss Bien of the Department Library indexed vol. 26, and the Chairman of this committee, vols. 27 and 28: It will be noted that almost exactly the same amount of index space per title was used in these three volumes, although the indexing system for vol. 26 differed from that of vols. 27 and 28. Preceding volumes were indexed by parcelling out the individual issues to different pathologists and librarians, so that much variation occurred and was to be expected. Furthermore, the number of entries required per paper depends to a large extent on the character of the contribution—moreso often than on its length: e.g., the published abstracts require almost and sometimes quite as much indexing space as the full papers; and papers with many organisms or new hosts take a correspondingly greater space to index than those concerned with one or only a few phases of a disease of one host. After looking over some of the extremes in index space used it was concluded that perhaps the closest estimate might be obtained by finding the average for the 28 volumes: this figure comes out as slightly over 9 titles per index page, while the average of index pages per volume is 20.2 (the first 7 volumes had only 6 issues each; there were 567 index pages in the 28 volumes, and 5217 titles—exclusive of errata and Society reports—to be indexed). Allowing 9.2 titles per index page for the 5217 titles we get a total of 567 pages for the 28 volumes, or 20.2 index pages per volume: for 30 volumes this would become 606 pages. In a cumulated index there is a good deal of combining of entries, so it is believed safe to assume that the 30-volume index would not take over 400 pages and very probably less.

A number of individuals agree that the cost of hiring a professional indexer would be prohibitive, and that a professional indexer—unless at the same time a plant pathologist—would do an inadequate job. On the basis of 11 years' experience as associate editor of *Biological Abstracts*, including a hand in working out the index system used and in training indexers, the chairman fully believes that with a carefully

## PHYTOPATHOLOGY

Vol.	Year	Pages text	Pages index	Number of				Titles indexed	Titles per index page	Av. pp. per title	No. of issues
				Papers	Absr.	Reviews	Notes				
1	1911	204	4	37	17	5	9	59	14.7	3.4	6
2	1912	276	11	41	37	4	9	91	8.3	3.0	
3	1913	313	13	44	31	4	17	96	7.4	3.2	
4	1914	417	16	35	100	2	21	158	9.9	2.7	
5	1915	356	9	52	20	1	15	88	9.9	4.0	
6	1916	454	10	47	69	5	33	154	15.4	2.9	
7	1917	458	11	49	63	4	28	144	13.1	3.2	
8	1918	627	14	59	..	7	36	92	6.6	6.8	12
9	1919	587	11	55	..	..	19	74	6.7	7.9	
10	1920	554	12	57	61	2	19	139	11.6	4.0	
11	1921	516	20	69	98	1	42	210	10.5	2.4	
12	1922	585	15	70	147	..	15	232	15.5	2.5	
13	1923	562	22	69	100	..	22	191	8.7	2.9	
14	1924	588	21	61	150	..	26	237	11.3	2.5	
15	1925	809	19	86	84	3	21	194	10.2	4.6	
16	1926	1012	19	78	95	4	23	200	10.5	5.0	
17	1927	836	21	79	65	3	12	159	7.6	5.2	
18	1928	1030	27	75	115	3	25	218	8.0	4.7	
19	1929	1147	26	88	95	2	55	240	9.2	4.7	
20	1930	1011	21	89	117	4	33	243	11.6	4.1	
21	1931	1207	22	90	96	6	21	213	9.7	5.6	
22	1932	1002	29	74	105	5	37	221	7.6	4.5	
23	1933	1006	28	78	120	7	38	243	8.7	4.1	
24	1934	1318	52	97	118	9	72	296	5.7	4.4	
25	1935	1118	43	81	165	6	36	288	6.7	3.9	
26	1936	1160	22	93	91	7	37	228	10.3	5.1 C'Bn	
27	1937	1186	21	88	88	5	36	217	10.3	5.4 FVR	
28	1938	939	28	99	139	7	47	292	10.4	3.2 FVR	

worked out set of directions—including examples—and a judicious selection of invited personnel we can enlist pathologists to do a volume or two each (the early 6-issue volumes each to count as half a volume). After 3 years' indexing on PHYTOPATHOLOGY, it is estimated that each issue would average 4 or 5 hours. Again, taking the experience of Biological Abstracts and Chemical Abstracts, it is believed that an honorarium of \$25.00 per each 12 issues would do the trick. For 50 to 60 hours' work \$25.00 may seem rather small pay for scientifically trained people, BUT the experience of these two journals has proved that an honorarium, however inadequate, serves as the added impetus needed to activate a person who is already interested in the subject. If the analytical-type of subject index used by Biological Abstracts—and the one used in PHYTOPATHOLOGY now for the third year—is employed it will be necessary to index anew only through volume 26, the index cards beginning with vol. 27 having been saved. Counting two of the 6-issue volumes as one, this leaves 22.5 "volumes" to be indexed; at \$.25.00 each, this would come to \$562.50.

For 13 years, Biological Abstracts has been using perforated sheets for typing the index entries. These are easy to type and handle, can be edited against the original papers readily before tearing apart into the individual 10 cards each, can be easily and rapidly marked for the printer and sent without copying, if so desired (thus making for accuracy), and there has never been any trouble with regard to printers' accepting them as copy. All the Botanical Abstracts and Biological Abstracts indexes have been sent in this way, and several other printers, including the Government Printing Office, have accepted this system without question. By numbering in final alphabetized order with a numbering machine assurance may be had that none of the entries are skipped. Biological Abstracts has never numbered them and, so far as we know, no entries have been lost.

This is probably not the time for a detailed discussion of indexing policies, but the general analytical type used in Biological Abstracts and in the last three volumes of PHYTOPATHOLOGY is preferred by the Committee; main subject words are alphabetized flush with the margin, and each subentry is inset 1, 2, or 3 places from the margin,

respectively. This form is most easily used, enabling the reader to see at a glance what is there. It may take somewhat more space than the "block system" formerly used in PHYTOPATHOLOGY, but this is more than made up for by the change in the author index and reduction of entries to simplest terms. Through volume 20 the entire titles of papers followed the authors' names in the index: this seemed unnecessary, since subject matter is all given first-place entry in the index. In the current index each author is given only first-place entry, joint authorship being indicated by the page reference in "(—)." Thus, John Doe and Henry Brown occur in the index as: "Doe, John, 22"; "Brown, Henry, (22)." New taxonomy, as in the past, is in blackface type.

Should the Cumulated Index program be approved, a detailed set of directions will be worked out and distributed to the invited collaborators doing the indexing. In the current volume, the Chairman has endeavored to follow the general lines approved by this Committee. However, since the Society has endeavored recently to enlist other than professional phytopathologists in subscribing to its journal, an effort has been made to present the material in such manner as to make unnecessary any knowledge of scientific names, though the latter also have been included. In a cumulated index it would be less readily feasible to go to quite this detail. All such matters will, of course, receive full consideration when or if the project is approved.

The Committee's estimates on the cost of preparing the proposed 30-volume index for the printer follow:

Honorarium at \$25.00 per each 12 issues to the indexers (index cards of Chairman's indexing saved for vols. 27-29, and will be also for 30) through 1936	\$562.50
Alphabetizing cards and typing	100.00
Honorarium for final editing (at least a two-months' full-time job)	200.00
Correspondence (postage on the index included in printer's estimates)	25.00
Index paper at \$2.50 per 1000 in 5000 lots, like sample (5000 should be ample, with margin of safety)	12.50

Total \$900.00

With an added \$100.00, making it an even \$1000, for safety, the total cost would then be this amount plus the printer's estimates, which follow:

"The per page price can stand exactly as it is. If you have more or less pages an adjustment can be made, or if we see the copy we shall be glad to make a bid on the whole manuscript exactly as it is submitted to us. We, of course, shall be glad to bill the Society on the per-page basis according to how many pages the manuscript may run to. This seems like a more satisfactory way of handling it, but we can make an estimate and abide by it, though we would then have to allow a margin of safety, but if it is done on the per-page basis there is no gamble on either side.

"Based on the usual index in *Phytopathology* we estimate the total price would be as follows:

	1,000 Copies	1,900 Copies	Weight each
Phytopathology stock	\$2,226.25	\$2,600.81	2½ lbs.
Science stock	2,092.00	2,347.81	2 lbs.
Biological Abstracts stock	2,107.50	2,377.31	1½ lbs.

(Book rate—1½ cts. per lb. in U. S. A.)

"You will note above that we have also given the weight of each book. Two-and-a-quarter lbs. would cost 4½ cts. to mail, while the Biological Abstracts paper would only cost 1½ cts. However, the penalty charge on thin paper would be a great deal more than the increased postage charge based on the weight of the books. As long as the book rate is in effect I think it is best that you use paper which is used in *Science*, sample of which you will find enclosed. We are in a position to give you this paper at a somewhat reduced cost because we buy it in large quantities for *Science* and other publications. We are giving you on a separate sheet the details showing how we arrived at the total cost." [Excerpts from letter of November 30, 1939, from Jaques Cattell, Vice-President and Secretary, The Science Press Printing Company.]

As above detailed, the estimates are for editions of 1000 and 1900, respectively; perhaps an intermediate number might more nearly fit the case. The Committee favors an edition of 1500 and the paper stock used in *Science*. A suggested price to Society members might be tentatively set at \$3.50 for prepublication and \$3.75 for post-publication orders, with \$3.75 and \$4.00, respectively, as the prices to nonmembers, though higher figures and a wider price spread might be desirable. Possibly announcement of exact prices and paper stock should be deferred until later.

If the green light is given by the Council, indexing work on the published volumes of PHYTOPATHOLOGY should go forward immediately after January 1, 1940, in order that the index cards for the first 29 volumes may be prepared, alphabetized, and edited so far as possible before the end of the year. During the year, as the issues appear, the

indexing for volume 30 can be done in duplicate for use (1) in the 1940 current volume index and (2) for interpolation in the cumulated index file as the work goes on. In this way the completed Cumulated Index should be ready to go to the printer in January, 1941; the date of publication after that would depend on promptness of printer and proofreaders.

The Committee agrees that Society members and subscribers should be circularized by double post card (with blank order or pledge to be signed on the return part), and that a full-page announcement on Cumulated Index plans be published in *PHYTOPATHOLOGY* at an early date. The only question is whether the *circularization* should be done immediately on approval of the project, or should await partial fulfillment of the work, when the number of pages, costs involved, and prices to be charged can be more definitely estimated.

The Committee feels that the advancing of necessary funds for carrying out the preparation of this index might well be placed in the hands of the Committee on Donations and Legacies of the Society. It is believed that such use would be entirely within the original objectives of the Lyman Fund, and that such use of any other funds in the hands of this Committee would be entirely proper and at the same time furnish a good example to the Society of the value of having such moneys available for its use. It is, therefore, suggested that the Council take up this matter with the Committee on Donations and Legacies and if agreeable to all concerned that this Committee be authorized to have charge of financing the Cumulated Index project, to be reimbursed by moneys received from the sale of the Index.

At this point it will probably have been surmised that the Committee recommends the preparation of a Cumulated Index for the first 30 volumes of *PHYTOPATHOLOGY*, and such is the case.

Respectfully submitted to the Council of the American Phytopathological Society.

FREDERICK V. RAND, Chairman  
GEO. L. PELTIER  
NEIL E. STEVENS

#### Supplement

##### Printer's Estimate on the Cost of Printing *PHYTOPATHOLOGY* Index

1,000 Copies, 8 pt. on 8, per page	\$ 5.48
1,900 Copies, 8 pt. on 8, per page	6.35
Additional 1,000 Copies, per page	.97
1,000 Covers and Covering	24.25
1,900 Covers and Covering	41.80
Additional 1,000 Covers and Covering	19.50

##### Cost of Printing 1,000 Copies, 400-page Index (*PHYTOPATHOLOGY* Stock):

400 pp. 8 on 8 at \$5.48	\$2,192.00
Covers and Covering	24.25
Mailing at \$10.00 M	10.00

\$2,226.25

Price Using *Science* Stock 2,092.00

Price Using Stock similar to *Biological Abstracts* 2,107.50

##### Cost of Printing 1,900 Copies, 400-page Index (*PHYTOPATHOLOGY* Stock):

400 pp. 8 on 8 at \$6.35	\$2,540.00
Covers and Covering	41.80
Mailing at \$10.00 M	19.00

\$2,600.80

Price Using *Science* Stock 2,347.81

Price Using Stock similar to *Biological Abstracts* 2,377.31

**The Sixth Pacific Science Congress.** The Sixth Pacific Science Congress was held at Berkeley, Stanford University, and San Francisco, California, July 24 to August 12, 1939. For the first time in the history of these congresses, plant pathology was represented by a definite subsectional program (Section VI, Botany, Subsection VI-B, Phytopathology). The program was organized by E. C. Stakman, Chairman. The meetings were held at the University of California, Berkeley, on August 4 and 5. The general topics were Virus Diseases of Plants, Variation in Plant Pathogens, Dissemination and Distribution of Plant Pathogens, and Plant Diseases Caused by Nutrient Deficiencies. Among those who contributed to the programs and discussions were Eubanks Carsner,

G. O. Ofcemia (University of the Philippines), T. E. Rawlins, J. M. Wallace, C. W. Bennett, W. C. Snyder, H. N. Hansen, J. H. Craigie (Winnipeg), José Vallega (Argentina), Hidenumi Asuyama (Tokyo Imperial University), H. S. Fawcett, H. R. McLarty (Summerland, B. C.), Kenneth Smith and V. H. Blackman (Cambridge, England), and H. L. Lyon (Honolulu). A total of 43 were in attendance.

At the close of the session on Virus Diseases of Plants, Eubanks Carsner, Chairman, the following resolution directed to the International Committee on Virus Nomenclature was adopted:

This Section favors the use in any system of plant-virus nomenclature of the Latin generic name of the host rather than the vernacular name, and the omission of the specific name of the host.

**Report of the Representative on the International Union of Biological Sciences.** Conditions in Europe have caused postponement of the scheduled 1940 meeting of the Union of Biological Sciences, which was to have been held at Stockholm in July. At this date, no prediction can be made as to when this meeting will take place. No proposals have been received by your representative the past year either from officers, committees, or individuals of our Society.

A. G. NEWHALL

**Report of the Delegates of the Congress for Microbiology Held in New York, N. Y., September 2 to 9, 1939.** This was the first Congress for Microbiology in which phytopathologists and mycologists were given an opportunity to meet pathologists and mycologists specializing in diseases of animals and human beings. Section VI (Fungi and Fungous Diseases) was permitted to hold five full morning programs and three joint programs with other Sections. While many of the 112 papers scheduled in these eight programs dealt with purely microbiological and physiological subjects, nevertheless they were all of fundamental importance to plant pathologists. Section III (Virus and Viral Diseases) scheduled 124 papers, many of which were of marked importance to phytopathologists.

In the symposium on Host-Parasite Relationships, dealing with (1) Etiology and Pathogenesis, (2) Tissue Reactions, and (3) Natural Resistance Including Immunity, G. H. Coons, J. C. Walker, and E. C. Stakman discussed the topics as plant pathologists, while Drs. Fred D. Weidman, D. J. Davis and J. G. Hopkins discussed them from the standpoint of medical pathology.

A. J. Riker cooperated with Michael Levine in organizing the program of 12 papers on the "Effects of Microorganisms and Chemicals on Atypical Growth in Plants."

A joint program was held with Section I (Variation and Taxonomy) on "Classification of Actinomycetes and Higher Fungi."

Professor L. R. Jones, as Dean of American Phytopathologists, was elected one of the five honorary presidents of the Congress, the only one from the United States. E. C. Stakman, C. L. Shear and A. H. Reginald Buller were elected Vice-Presidents of Section VI. G. M. Reed and Michael Levine served on the local committee of arrangements for Section VI and other members assisted. Sixteen hundred persons registered officially at the Congress. The Proceedings will include the addresses given at the general sessions of the Congress, published in full, and abstracts of over six hundred papers presented on the programs.

B. O. Dodge served on the Executive Committee of the Congress and as Convener of Section VI (Fungi and Fungous Diseases). He addressed a general session on "Some Problems in the Genetics of Fungi."

The Fourth International Congress for Microbiology is scheduled to meet in Copenhagen in 1942. It is recommended that the Society take an active part in the Congress.

B. O. DODGE and WM. H. MARTIN

**Report of the Resolutions Committee.** 1. RESOLVED that The American Phytopathological Society express its appreciation to the A. A. A. S. committees responsible for the arrangements that have contributed so effectively to the success of the 1939 meeting in Columbus.

2. RESOLVED that The American Phytopathological Society convey to the management of the Neil House expression of gratitude for the courteous and efficient service extended to the members attending the thirty-first annual meeting. This resolution was enthusiastically adopted by a rising vote.

3. RESOLVED that, on behalf of The American Phytopathological Society, we express our appreciation to the various local agencies and committees for their many courtesies and most efficient services; to the Governor, the honorable John W. Bricker, for a delightful tea arranged for our guests; to The State Department of Visual Instruction and the Spencer Lens Company for supplying us with projection equipment; to the troop

of Boy Scouts of America for messenger service during the sessions; and to the City School Teachers of Columbus for their help in operating the projectors.

4. RESOLVED that, on behalf of the members of our Society, we express to our officers and committee members our deep appreciation for their untiring efforts in furthering the best interests of our Society.

5. RESOLVED that, on behalf of the Society, we express to our committee on arrangements for the Columbus meeting, W. G. Stover, chairman, A. L. Pierstorff, C. C. Allison, Harry Atwood, H. C. Young, and P. E. Tilford, and A. J. Riker and the sub-committee on exhibits, our gratitude for their very substantial contribution to the success of the meeting, and to the Department of Plant Pathology of the University of West Virginia, particularly Genevieve Clulo, Eldor Martin, William Dorrell, and Donald Hoffmaster, for entertaining us so delightfully during the dinner.

6. RESOLVED that, on behalf of the Society, we express to Professor L. R. Jones our deep appreciation for his thoughtfulness and generosity in donating to the Society five hundred separates of his memoir on the life of Dr. Erwin F. Smith.

G. W. KEITT  
M. W. GARDNER  
G. H. COONS

#### ACTION BY THE SOCIETY AT THE 1939 COLUMBUS MEETING

**Elections and Appointments.** The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Sixty-two applicants were elected to membership.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: J. S. Boyce, C. W. Bennett, and R. P. White.

**Reports of Officers, Representatives, and Committees.** The reports for the year 1939, as presented on previous pages, were read and accepted.

**Recommendation Regarding Abstracts.** The Society confirmed the Council's recommendation that the Secretary be allowed to copy the titles of all abstracts before they go to the Editorial Committee and make up the program from that material, so that the program may be in the mail early; and, that the Secretary be notified by the Editorial Committee of any rejections of abstracts. (Papers received after November 1 will be rejected.)

**Committee on Nomenclature and Classification of Plant Viruses.** The Society confirmed the Council's recommendation that the temporary committee on virus nomenclature be made a standing committee.

**Committee on Publicity and Public Relations.** The Society confirmed the Council's recommendation that the temporary publicity committee be made a standing committee, Publicity and Public Relations.

**Committee on Regulatory Work and Foreign Plant Diseases.** The Society confirmed the Council's recommendation that, since at the request of the chairman of the committee that all members of this committee in the United States be dropped, the Committee on Regulatory Work and Foreign Plant Diseases be discharged.

**Committee on Research Monographs of the Association of Land-Grant Colleges and Universities.** The Society confirmed the Council's recommendation that the incoming President appoint a temporary committee to study and make a report as soon as possible on the request of the committee on Research Monographs of the Association of Land-Grant Colleges and Universities.

**Committee on Terminology of Immunology and Use of Technical Words.** The Society confirmed the Council's recommendation that the Committee on Terminology of Immunology and Use of Technical Words continue to be temporary, and the report be printed in PHYTOPATHOLOGY in the Annual Report.

**Constitution and Standing Rules.** The Society confirmed the Council's recommendation of the publication of the Constitution and Standing Rules.

**Cumulated Index of PHYTOPATHOLOGY.** Upon recommendation of the Council, the Society voted to go on record as endorsing in principle the report of the committee, and instructing the President-elect to make any appointments or changes he deemed necessary for the committee to work out the details of the plan.

**Summer Meeting.** The Society, on Council recommendation, voted to hold a summer meeting at Seattle, Washington, in connection with the A. A. A. S. summer meeting, June 17-22, 1940, and that arrangements be in charge of the Pacific Coast Division of the Society.

**The 1940 Annual Meeting.** It was recommended by the Council, and voted by the Society, that the time of the annual meeting in 1940, at Philadelphia, Pennsylvania, be from the morning of December 27 (Friday) to December 31 (Tuesday), with the Sunday intervening to be used for informal conferences.

**Council Policy.** The Council expressed the desire to state that it is at present the policy of the Council, and they approve of the idea, of changing the appointments on committees often, thus putting them on a rotating basis.

**Council Recommendation.** The Council reported that the Society has been helping the Central Bureau of Schimmeleutures by giving them advertising space in the journal. In order to help the Bureau financially, the Council urged members to buy cultures from this Bureau as they are needed.

**IVAN CLAUDE JAGGER**

August 12, 1889—February 16, 1939

Ivan Claude Jagger was graduated from Cornell University in 1911 with the degree of Bachelor of Science in Agriculture, and in 1913 he was granted the degree of Master of Science by the University of Wisconsin.

From 1911 to 1913, Mr. Jagger was an Industrial Fellow, and 1913-14, Instructor in Plant Pathology at Cornell University; from 1914 to 1918 he was Assistant Professor of Plant Pathology in the College of Agriculture, Cornell University, and Instructor in Biology at the University of Rochester; from 1918 to the time of his death he was Pathologist, and in later years Senior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, except during 1926 when he held a fellowship with the International Education Board.

Mr. Jagger achieved outstanding success along a number of lines, especially in breeding high-quality lettuce and melons resistant to mildews and certain other diseases. He possessed a most pleasing personality, combining thoughtfulness, congeniality, and genuineness. He had many friends and all regarded him most highly.

### LAWSON MERRILL HILL

March 28, 1909-May 6, 1939

Lawson Merrill Hill was graduated from West Virginia University in 1935 with the degree of Bachelor of Science in Agriculture, and in 1937 he was granted the degree of Master of Science by the same institution.

From 1936 to the time of his death, he was research Assistant in the Department of Plant Pathology and Bacteriology of the West Virginia Agricultural Experiment Station.

Mr. Hill was a student and investigator of much promise. He was especially interested in the microchemistry of pathological tissues. He was one of the most popular students in his university class and won high honors in many fields of activity. His friendly nature and pleasing sense of humor endeared him to all his associates.

## ROLAND ELISHA STONE

November 4, 1881-June 4, 1939

Roland Elisha Stone was graduated from the University of Nebraska in 1906 with the degree of B.Sc. In 1908 he received his M.Sc. from Alabama Polytechnic Institute and his Ph.D. was conferred by Cornell University in 1913. At Cornell University he was also elected a member of the Sigma Xi.

From 1912 until 1917, Dr. Stone was Lecturer in Botany at the Ontario Agricultural College, Guelph, Canada, and in 1917 he became Associate Professor of Botany, which position he held until his death. He spent twenty-seven years in the service of the Ontario Agricultural College.

He was a sustaining life member of The American Phytopathological Society, a member of the Canadian Phytopathological Society, the Botanical Society of America and a Fellow of the American Association for the Advancement of Science.

Dr. Stone's chief interests were in teaching, in which he excelled, and in research in the field of plant pathology and mycology. Among his many achievements were the selection of varieties of canning peas resistant to root rot and suitable to the Ontario Canning Industry, and his publications on the edible and poisonous mushrooms of Ontario.

He was an enthusiastic botanist with high ideals, a keen sense of duty and the ability to inspire and direct students in their search for scientific knowledge. He had a quiet unobtrusive manner and a kindly personality combined with a dry sense of humour. These qualities endeared him to all who became well acquainted with him. His outstanding characteristic, however, was his spirit of loyalty to his profession, his College, his colleagues, and his friends.

## EDWARD JACOB PETRY

June 24, 1880—October 8, 1939

Edward Jacob Petry was graduated from Ohio State University, with the degree of Bachelor of Science, in 1907. In 1914, he received the degree of Master of Science from Purdue University, and in 1925, the degree of Doctor of Philosophy from Michigan State College.

From 1907 to 1910, he served as Assistant in Botany, Cornell University; from 1911 to 1916, he was Instructor in Agronomy, and 1916 to 1918, Assistant Professor of Agricultural Botany, Purdue University; from 1918 to 1920, Instructor in Botany, University of Michigan; from 1920 to 1923, Professor of Botany, South Dakota State College; from 1923–1924, Consulting Botanist, South Dakota Experiment Station; 1924–1925, Survey Botanist, South Dakota Geological and Biological Survey; from 1926 to 1929, Professor of Biology, Hendrix College; from 1929 to 1931, Professor of Biology, Central College (Mo.); from 1931 to 1933, Professor of Botany and Associate in Physiology, Coe College; from 1933 to 1935, Consulting Biologist and Chemist, Cedar Rapids Water Works; 1936, Chemist, Consumers Co-operative Association, Kansas City, Mo.; 1937 to time of his death, Paint Chemist, Ebony Paint Co., Kansas City, Mo.; also, 1939 to time of his death, Professor of Chemistry and Medical Technologist, Central College of Osteopathy, Kansas City, Mo.

Dr. Petry was a life member of The American Phytopathological Society and had been a member of a number of other scientific societies. He had a naturally inquiring mind and was always anxious to ferret out the unknown in any situation. He had a very friendly disposition, and aimed always to be helpful to those in distress and to practice the "Golden Rule."

## ERRATA, VOLUME 29

Page 75, table 2, column 1, *read* Feb. 10 *for* Feb. 11; column 4, *read* Feb. 11 *for* Feb. 10.

Page 92, line 12, *read* sedimented less rapidly *for* sedimented more rapidly.

Page 658, line 12, *read*  $\frac{p}{1-p}$  *for*  $\frac{1}{1-p}$ .

Page 908, line 8, last paragraph, *read* hood *for* blood.

## PRELIMINARY ANNOUNCEMENT

The American Phytopathological Society will hold its summer meeting at the University of Washington, Seattle, Washington, June 19-22, in connection with the regular meetings of the Pacific Division of the Society.

The program will include a general meeting of horticulturists, entomologists, and plant pathologists, a symposium on fruit tree virous diseases, an all-day field trip to the Western Washington Experiment Station at Puyallup, and three half-days for the presentation of papers.

Plant pathologists who wish to present papers at this meeting should send their titles, prior to May 1, to L. D. Leach, Secretary-Treasurer, Pacific Division, Davis, California.

# CULTURAL AND GENETIC STUDIES ON USTILAGO ZEAE<sup>1</sup>

C. G. SCHMITT

(Accepted for publication January 5, 1940)

## INTRODUCTION

These studies were initiated to determine the nature of inheritance of factors for characters in *Ustilago zeae* (Beckm.) Unger, and to arrive at a better understanding of the phenomenon of sectoring in culture. Inbreeding was undertaken to establish uniform lines with distinctive characters satisfactory for crossing and favorable for the study of induced mutation.

*Ustilago zeae* is a desirable species for use in the study of the genetics of fungi because its life cycle is relatively short (as many as 10 generations may be grown in a single year); it is a heterothallic species with distinct haploid characters; each chromatid can be sampled; and the fungus is widely distributed, available in quantity, and readily cultured.

## METHODS OF SINGLE-SPORE ISOLATION AND OF INBREEDING

Monosporidial lines were established by isolating sporidia from promycelia with a modified Chamber's micro-manipulator. Dickinson's (7) and Hanna's (13) techniques were followed with the exception that the sporidia were transferred immediately to agar slopes by means of a flat-tip transfer needle. Germination of chlamydospores occurred in from 12 to 24 hours on Czapek's agar at 25–30° C. At lower temperatures germination was delayed and a typical promycelium was not always formed. These findings confirm those of Hüttig (16). The spores exhibited no period of dormancy. High germination was obtained from spores removed from galls that were not entirely dry. A discussion of the literature on dormancy of spores of this species has been presented by Stakman (24).

The system of culture numbering employed by Christensen (4) was used. Inbreeding was practiced for 10 successive generations by introducing by hypodermic needle all possible combinations of cultures of monosporidial lines from the same promycelium in pairs into susceptible corn plants. Ten plants usually were inoculated with each cross. Cultures of individual lines also were inoculated into plants to determine the extent of monopathogenicity in the isolates. In preliminary tests, plants of all ages were inoculated. It was found that infection occurred when the sporidia were in contact with meristematic tissue, irrespective of the age of the plants. Plants in the greenhouse were inoculated when a week old, and mature galls were harvested 3 to 4 weeks later. The greenhouse temperature was held at 27–32 degrees C., because it was found that gall formation occurred within this range but did not occur below 21° C. Tisdale and Johnston (27) found that seedlings in the greenhouse were as susceptible

<sup>1</sup>A portion of a thesis submitted to the Department of Botany, University of Missouri, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

to infection as were older plants. They emphasized the necessity of taking cognizance of the relation of temperature to infection. Plants grown at low temperatures inoculated with compatible lines usually developed anthocyanin at the point of inoculation. This phenomenon was not observed in plants inoculated with incompatible lines.

#### MEDIA

During the first 3 generations of inbreeding the following media were used for comparison of cultural characteristics: Modified Czapek's ( $MgSO_4$ , 0.30 g.;  $KH_2PO_4$ , 1.25 g.; KCl, 0.50 g.;  $FeSO_4$ , 0.01 g.; Asparagine, 1.00 g.;  $NaNO_3$ , 0.50 g.; sucrose, 30.00 g.; agar, 15.00 g.; and distilled water to 1 liter), malt-extract agar, Carter's, potato dextrose, Difeo corn-meal agar, and a medium (referred to in this paper as "S"), used by Stakman *et al.* (25), which consisted of: sucrose, 3%;  $Ca(NO_3)_2$ , 0.006%; and agar, 1.8%. Subsequent generations were grown upon Difeo corn-meal agar. To compare the cultural characters of the first generation with those of any succeeding generation, the lines to be compared were inoculated on a given date into 125 ml. flasks containing 35 ml. of the same lot of medium. By following this procedure the fluctuations in characteristics of a line that might result from slight variations in the constitution of 2 separately prepared lots of a given medium were avoided. Cultures were then incubated at 25-27° C. Stock cultures were maintained on modified Czapek's agar in 6-inch test tubes at 10-18° C. to prevent sectoring.

#### EFFECT OF THE MEDIUM ON SECTORING

The effect of the medium on the rate of sectoring of this fungus has been studied in some detail by Stakman *et al.* (25). They found that the kind of medium used affected mutation rate. Table 1 summarizes the influence of

TABLE 1.—*Effect of the medium on rate of sectoring in Ustilago zaei.* Twenty monosporidial lines were grown in duplicate on each medium. Results were recorded following 3 weeks' incubation at 25-27° C.

Medium	Number of sectors
Difeo corn-meal agar	68
Modified Czapek's agar	79
Malt-extract agar	90
S	94
Potato-dextrose agar	106
Carter's medium	107

the media on frequency of sectoring. On the basis of these results, modified Czapek's was selected as a desirable medium for the maintenance of stock cultures, since the rate of sectoring on it was relatively low and the rate of growth good. Difeo corn-meal agar was selected as the medium to detect mutation following irradiation because of its uniformity and the low rate of sectoring exhibited upon it.

When a given line of this fungus is grown on a medium of one composi-

tion, it may exhibit an appearance at variance with that displayed when it is grown on another medium, but if transferred from the second medium back to the first the appearance of the colony will be identical with that originally shown on the first medium. No gradual change in type has been observed in any case. These observations are in conformity with those of Stakman *et al.* (25).

#### EFFECT OF IRRADIATION ON SECTORING

That ultraviolet radiation is sometimes a means of inducing mutation in fungi has been demonstrated by a number of investigators. Stevens (26) reported ultraviolet radiation effective in inducing perithecial formation in *Glomerella cingulata* (Stoneman) Spaulding and Schrenk and pycnidial formation in *Coniothyrium* sp. Greaney and Machacek (12) produced a white, fertile saltant of *Helminthosporium sativum* P. K. and B. by ultraviolet treatment. Hollaender and Emmons (15) and Emmons and Hollaender (10) produced variants in *Trichophyton mentagrophytes* (Robin.) Sab. with monochromatic ultraviolet radiation. The new type colonies of this fungus differed from the normal in form, color, growth rate, and spore production. The wave lengths most effective in killing, 2650 to 2537 Å, also were most effective in production of variants. Dickson (8) found that mutations induced in *Chaetomium cochlioides* by X-rays did not differ essentially in their characters from those induced by ultraviolet treatment. Seven other species of fungi did not respond to irradiation when subjected to the same treatment. He induced saltants in both plus and minus strains of *Phycomyces blakesleanus* Burgeff by exposing them to X-rays.

Twenty-four-hour-old broth cultures of sporidia to be irradiated with ultraviolet or X-rays were spread in a single layer on the surface of Czapek's agar on double-width micro slides, according to Landen's (19) method. Ultraviolet treatments were applied to sporidia of the 5th inbred generation using the 2537 Å line and dosages ranging from 2500 to 6800 ergs/mm<sup>2</sup>. The results are summarized in table 2.

TABLE 2.—Comparison of rate of sectoring in colonies from sporidia irradiated with ultraviolet light (2537 Å) of various dosages. Results after 5 weeks' incubation on Difco corn meal agar

Line	75: 2 (sporidial)			75: 3a (mycelial)		
	Dosage ergs/mm <sup>2</sup> .	Total no. colonies	No. of colonies with sectors	Percentage of colonies with sectors	No. of colonies	No. of colonies with sectors
0	147	107	72.8	134	28	20.9
2,500	60	52	86.7	63	15	23.8
4,000	99	71	71.7	50	9	18.0
5,000	245	177	72.2	131	54	41.2
6,000	39	23	59.0	83	26	31.3

From 2 days to a week after treatment the colonies developing from these treated sporidia were transferred to 125 ml. culture flasks containing

Difco corn-meal agar. Although over 770 such colonies were observed for five weeks following transfer, the rate of sectoring in the colonies from treated sporidia was not in excess of that for the check. The mutation rate apparently was not affected by ultraviolet treatment.

Monochromatic irradiation was applied<sup>2</sup> to 5th inbred generation chlamydospores, using the 2650 Å line and dosages ranging from 21,000 to 56,000 ergs/mm<sup>2</sup>. There was a noticeable delay in germination of treated spores and the delay increased with the dosage applied. Those given 56,000 ergs/mm<sup>2</sup> germinated 60 hours after transfer to media. The lethal effect also increased with the dosage. Over 40 sets of sporidia were removed from treated spores, but no indication of mutation induced by radiation was found in the colonies that developed from these sporidia.

The highest X-ray dosage used was approximately 4500 r units. This was not sufficient to exhibit any lethal effect. Only 150 colonies from treated sporidia were observed, but there was no indication of mutation in them.

Failure of irradiation to affect the mutation rate of this fungus strengthens the conclusion drawn by others; e.g., Heldmaier (14), Matsuura (21), Leonian (20), and Christensen and Graham (5), that unstable species are not necessarily more unstable following irradiation. Borzini (3) treated spores of *Ustilago zea* with ultraviolet after germination was initiated. The promycelia and sporidia produced were shorter and thicker than those of the checks. Other than this no apparent changes were produced.

#### EFFECT OF TEMPERATURE ON SECTORING AND GROWTH

Some workers have been successful in producing mutations by applications of heat to fungi. Barnes (1) found that exposures of the spores of *Eurotium herbariorum* (Wigg.) Link, for two minutes at temperatures of 60–80° C. produced mutation. In a later work (2) similar results were obtained with *Botrytis cinerea*. Dickson (8) applied heat treatments near the maximum to *Chaetomium cochliodes* but noted no mutants as a result of the treatment.

In an attempt to induce mutation by heat treatments near the thermal death point 12-hour old sporidial suspensions of a tenth-generation inbred line in malt extract broth were held at temperatures of 50, 55, 60, and 65° C. for 10 minutes. Poured plates were then made of the suspensions. The thermal death point was found to be somewhere between 55° and 60° C. Two days later individual colonies were removed from the 55° plates and transferred to agar slants. Although approximately 200 colonies developing from these sporidia were observed on agar plates, only 16 were removed for more extended observation. There was no indication of induced mutation.

The effects of temperature on the rate of growth and sectoring are presented in table 3. The fifth-inbred-generation lines used in this test were 75:2, a sporidial line, and 75:3a, a mycelial line. The results indicate that

<sup>2</sup> The writer is grateful for technical assistance to E. W. Landen of the Department of Physics of the University of Missouri.

the rate of sectoring is not necessarily highest at the optimum-growth temperature. In general, it may be stated that mycelial lines grow more rapidly than do sporidial lines at temperatures near and below the optimum. Stakman *et al.* (25) likewise found considerable variability between lines in their response to temperature.

TABLE 3.—*The effect of various temperatures upon the growth and rate of sectoring of inbred lines 75: 2 and 75: 3a of *Ustilago zeae*, after 4 weeks' of incubation. Cultures grown in quadruplicate*

Temperature	<i>U. zeae</i> No. of culture	75: 2 (sporidial)		75: 3a (mycelial)	
		Average diameter of colony mm.	No. of sectors	Average diameter of colony mm.	No. of sectors
°C.		mm.		mm.	
10	1	10	0	16	0
	2	9	0	17	0
	3	8	0	17	0
	4	8	0	16	0
15	1	13	0	35	0
	2	13	0	33	0
	3	12	0	32	0
	4	12	0	33	1
20	1	18	6	42	0
	2	21	5	41	0
	3	18	3	42	0
	4	15	7	42	0
25	1	21	14	43	0
	2	22	5	46	0
	3	19	10	47	0
	4	20	12	46	0
30	1	22	3	38	2
	2	20	5	38	3
	3	21	2	37	1
	4	23	0	37	0
34	1	20	0	15	0
	2	19	0	9	1
	3	19	0	13	0
	4	19	0	9	0
37.5	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0

#### EFFECT OF INBREEDING ON SECTORING

To compare the rate of sectoring of lines of the fungus that had been inbred to various generations, quadruplicate cultures of a number of lines were set up on corn-meal agar at a temperature of 25–28° C. (Table 4). Inbreeding failed to increase the stability of this stock of *Ustilago zeae*. There was a clear cut difference between the rate of sectoring of sporidial and mycelial lines but in each type the rate of sectoring appeared to be constant.

TABLE 4.—*The effect of inbreeding on the rate of sectoring in *Ustilago zeae* after 5 weeks' incubation at 25 to 28 degrees C.*

Sporidial lines				Mycelial lines			
No. of spore	No. of gen. inbred	No. of sporidium	No. of sectors	No. of spore	No. of gen. inbred	No. of sporidium	No. of sectors
500	0	1	15	500	0	2a.4	6
		2	18	700	0	4e.1	5
		3	24			1.1	7
		4	29			3e.4	3
700	0	4e.3	20	503	1	1.2	4
503	1	1	21	707	1	1.1	2
707	1	4	17			2.1	6
		1	26			3.1	5
		2	11	510	2	2.1	3
		1	19	716	2	1.1	4
510	2	1	19			2.2	7
716	2	1	20			3.3	4
512	3	1	25	512	3	3.6.1	5
		2	16			4.2	3
		3	18			1.1	6
		4	17			1a.3	2
514	4	1	10	514	4	3.1	5
		2	22			1b.1	3
		3	31	587	4	1	6
		4	13	593	5		
587	4	2	17			2	4
76	5	4	16	76	5	1	6
2003	6	2a	27	647	6	1	3
		1	23	671	7	1	1
		2	19	1072	8	2	5
				2069	9	1	3
				2079	10	3	4

#### ATTEMPTS TO INDUCE CHLAMYDOSPORE FORMATION IN VITRO

With a view to avoiding the necessity of inoculating the host plant, an effort was made to induce chlamydospore formation in pure culture. Some investigators have reported chlamydospores by some species of the Ustilaginales *in vitro*. Kniep (18), who obtained chlamydospores of *Urocystis anemones* (Pers.) Kniep non Winter, was the first to obtain the complete life cycle of one of the smuts *in vitro*. Chlamydospore production in one or more of the species of *Ustilago* has been reported by Fleroff (11), Sartoris (23) and Wang (28). Various compounds were added to a modified Czapek's agar and the media were inoculated with compatible lines. A number of concentrations of  $\beta$ -indole-3-acetic acid, indole-3-propionic acid, vitamin B<sub>1</sub>, vitamin C, pantothenic acid, and nicotinic acid were used. Extracts of host plants, sterilized by steam under pressure and by filtration, were added to agar. Shredded plant material also was mixed with agar. Compatible lines growing adjacent to each other were irradiated with ultraviolet light, using the 2537 Å line and a dosage of 4000 ergs/mm<sup>2</sup>. None of the methods tried was successful. Failure to obtain typical chlamydospores of *U. zeae* in pure culture by the methods employed in this study does not preclude the possibility of their formation. It is possible that some labile substance is present in the host that induces chlamydospore formation.

## INHERITANCE OF FACTORS

## a. Growth Type

Sporidial and mycelial types of growth have been described by Kernkamp (17) and by Stakman *et al.* (25). Isolations were made from 4 field collections of galls and in every case the isolates were sporidial in type of growth. The mycelial types studied in this investigation arose as mutations from sporidial-type colonies. They remained mycelial in type through successive subcultures. Examinations of microscopic mounts from colonies of mycelial type revealed a few sporidia among the hyphal segments. Although sporidia predominated in the sporidial-type colonies, a few short hyphae were found. Single hyphal segments isolated from sporidial-type colonies yielded colonies of that type. Likewise, single sporidia from mycelial-type colonies in turn yielded mycelial-type colonies. Crosses between monosporidial lines of sporidial type and mycelial type gave a preponderance of 1:1 segregations, but 3:1 and 1:3 ratios for both types were present. Crosses between mycelial lines always yielded mycelial types and sporidial-line crosses yielded only sporidial types. Nine complete sets of sporidia were isolated from a chlamydospore formed by crossing third-generation mycelial and sporidial types. Each of the sporidia from cells 1 and 2 gave rise to mycelial-type colonies; those from cells 3 and 4 produced sporidial-type colonies.

Sporidial-type colonies frequently formed sectors of mycelial type, but mycelial-type colonies never were observed to form sporidial-type sectors. They did, however, form other mycelial-type sectors.

A comparison of the rate of sectoring of colonies of the 2 types of the 5th inbred generation was made. Of 570 colonies of sporidial type allowed to grow for 5 weeks on corn-meal agar at 25–27° C., 430, or 75.4 per cent, formed sectors. Of 451 colonies of mycelial type, 132, or 29.3 per cent, formed sectors. From these results it appears that the mycelial-type colony is approximately 3 times as stable as the sporidial type.

## b. Sex

In the first 3 inbred generations bipolar sexuality existed, but in the 4th generation this was disturbed. Three of the 4 monosporidial lines from one spore were monopathogenic. The monopathogenicity of one of these lines was verified by making 3 single-spore isolations from the culture and through introducing these into plants. These inoculations produced galls, but sporidia isolated from the spores in these galls were not monopathogenic. Out of some 4,000 monosporidial lines only the 3 above-mentioned lines were monopathogenic. Christensen (4) and Eddins (9) found a higher percentage of such lines in their material.

## c. Color

An apparently uniform mycelial cream-colored (22) line was obtained in the 5th inbred generation from a cross between two similar mycelial

sectors. The 6th generation appeared uniform and identical with the 5th generation parents. The 7th generation, however, segregated beige and cream color. The sudden appearance of the beige color was perhaps due to the segregation of a modifier for color. Each of these types was established as a uniform line in the 8th generation. The beige-color line was inbred to the tenth generation.

#### SEGREGATION

Segregation of factors for sex, color, and type of growth occurred in either Division I or in II as determined by seriation of the promycelial cells. For each of these characters 1:1, 3:1, and 1:3 ratios were found and a 4:0 segregation was found in one case for sex. The 3:1 and 1:3 ratios may be due to delayed segregation, as has been shown by Dickinson (6) for *Ustilago avenae* and *U. levis*. To make certain of this at least three successive sets of sporidia should have been isolated from the spores in question.

#### MUTATION

The sectors and "patch mutants," which appear from time to time on colonies grown upon most laboratory media at ordinary temperatures, are regarded as mutations. Their uniformity in successive subcultures and the fact that they can be recovered intact following crosses indicate that they have a genetic basis. The writer agrees with Stakman *et al.* (25) that the so-called "losses" of virulence often reported in the literature are due to mutation. In the course of this investigation no gradual changes in characteristics were ever observed. The fact that sectors in turn gave rise to other sectors renders delayed segregation unlikely as an explanation for the phenomenon of sectoring.

#### SUMMARY

Spores germinated in from 12 to 24 hours on Czapek's agar at 25–30° C. At lower temperatures germination was delayed and atypical. Spores subjected to monochromatic ultraviolet irradiation exhibited delayed germination. The lag period increased with increases in dosages.

Chlamydospores exhibited a high percentage of germination immediately after collection, even though the galls were not always entirely dry. No indication of a "rest period" was ever found.

Plants of all ages were found susceptible to *Ustilago zeae* so long as meristematic tissue was present and the temperature was favorable to infection.

At temperatures below 21° C. no infection of the host was obtained with compatible lines in the greenhouse. There was, however, anthocyanin formation. At temperatures above 27° C. high infection was obtained.

The medium was found to exert a pronounced effect upon the appearance of colonies and mutation rate. Cultures on Difco corn-meal agar gave rise to fewer mutations than did those on more nutritive media. Czapek's agar was used to maintain stock cultures.

The measures employed to prevent mutation in stock cultures included

incubating them at temperatures below 18° C. on a medium low in available nutrients with frequent transfers to fresh lots of media.

Mutations were not induced by ultra-violet irradiation, by X-rays, or by brief exposure of sporidia to temperatures near the thermal death point.

Increases of incubation temperature within limits increased the frequency of mutation. Below 20° C. few, if any, mutations were found. Thirty-seven and a half degrees C. was found to be above the maximum for the lines studied.

The frequency of mutation was not affected by inbreeding for ten generations.

A number of unsuccessful attempts to induce chlamydospore formation of *U. zeae* in pure culture were made.

Stocks exhibiting the characteristic mycelial type of growth, sporidial type of growth, and beige and cream color were established. The sporidial and mycelial types of growth were established in the parental generation and inbred as far as the seventh and tenth generations respectively. Beige and cream colored lines were established in the eighth inbred generation and the former was inbred to the tenth generation.

The mutation rate of sporidial lines was approximately three times that of mycelial lines.

Segregation of factors for color, for sex, and for type of growth occurred in both I and II of the reduction divisions.

Three monosporidial lines out of some 4,000 were monopathogenic. Spores from galls produced as a result of inoculation with one of these lines did not give rise to monopathogenic lines.

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## ONION EELWORM ROT OR BLOAT CAUSED BY THE STEM OR BULB NEMATODE, *DITYLENCHUS DIPSACI*

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### REVIEW OF LITERATURE

"Eel-disease," "Kroefzickte," or onion "bloat," has been known in Europe since its first observance by Kühn (10), whence it may have come to America. Kühn named the causal organism *Tylenchus putrefaciens*. Beijerinck (1) called it *Tylenchus allii*, but Ritzema Bos (16, 17, 18) showed it to be the same species as that described from teasel (*Dipsacus*), narcissus, hyacinth, and rye. He succeeded in transferring the strain from onion to rye and from hyacinth and rye to onion. Ramsbottom (15) and others have transferred the narcissus strain to onion, and Walton (22) reported mass transfers of the onion strain to parsnip. He also found (23) the disease could persist in soil 4 to 5 years.

From the descriptions of the disease given by Chatin (3) in an early thesis in Paris, by Beijerinck (1) and by Ritzema Bos (18), it is known that the nematode may infect the bulb, the stem, leaves, flowers, and occasionally even a small percentage of the seeds, although diseased plants are said to go to seed rarely. Besides onion, Goodey (8) now lists shallot, leek, garlic, chives, and wild onion (*A. vineale*) as susceptible.

#### HISTORY OF THE DISEASE IN THE UNITED STATES

Although recorded by Bessey (2) as occurring on rye as early as 1907, and by Smith (19) as affecting red clover in the northwestern States 20 years ago, the first authenticated record of *Ditylenchus dipsaci* (Kühn) Filipjev on growing onions in this country according to Steiner (20) was an infestation that broke out in 1929 or 1930 on the muck farm of James Dellaquila near Canastota in Madison County, New York. To be sure, it had been found before that on ships' stores and is listed by McKay (12) not only as a serious pest on strawberry and clover in Oregon but also as occurring on onions somewhere in the United States. Godfrey and Scott (7) found it on garlic in California and recorded that this strain could attack salsify, parsley, and even celery. This brought the number of cultivated food plants known to be susceptible in America well above 30 out of some 195 hosts recently listed by Steiner and Buhrer (21).

In view of the apparent virulence of the attack at Canastota, where a one-crop system of muck farming is well entrenched, and of the devastation reported by Cobb (5) as occurring in New South Wales, where a similar situation existed, vigorous steps were taken to stamp out this infestation. This was done in 1931 by steaming about 15,000 square feet of muck with a pair of inverted pans operating on a 30-horse-power traction engine loaned by the Town of Canastota. Treatment was done between August 24 and 29, when the muck was in a favorable condition for quick penetration of the steam. Temperatures above 180° F. were obtained 6 inches below the surface after 20 minutes of steaming. The cost of this treatment, including construction of the two pans, labor, coal and piping (Fig. 1, A and B) was \$230.00, borne by the Bureau of Plant Industry of the New York State Department of Agriculture. The work was supervised and reported by the senior writer (13). Lettuce and celery were grown on this field the following year as further precaution. Numerous subsequent surveys during the next 8 years covering this and adjoining farms revealed no further trace of the disease. While it has not been reported recently on onion in other States, the reason simply may be due to no one having recognized it.

In 1938 this disease was again discovered on 3 farms a few miles distant from the Dellaquila field. The same appearance of poor stand, weak prostrate foliage, suggesting lightning injury (Fig. 2), followed late in the season (August 20) by much rotting and splitting of bulbs (Fig. 3), was in evidence. All growers reported that the trouble had been of several years' duration, worse some seasons than others, and definitely on the increase.

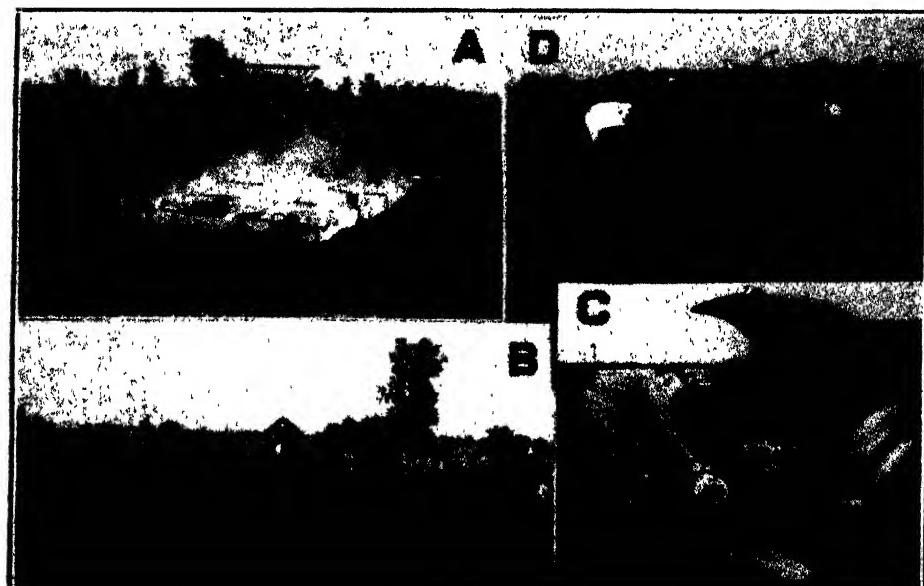


FIG. 1. Field views of eradication methods employed. A. Steaming at Canastota with inverted pan. B. Two infested areas covering nearly 15,000 sq. ft. were steamed in 6 days at a total cost of \$230. C. Three smaller areas have been treated as shown here by injecting chloropicrin at close intervals. D. Filling the injectors, showing method of handling chloropicrin in the field.

One grower had given up trying to raise onions on about one-fourth of an acre where the trouble was the worst.

Again, the New York State Bureau of Plant Industry under the direction



FIG. 2. Portion of a diseased area of Early Yellow Globe onions on muckland in Madison County, N. Y., taken August 13 (by R. L. Clement) showing thin stand, weak foliage, stunting and many dead outer leaves.



FIG. 3. Bulbs with rot caused by nematodes and associated injuries. A. A plant 14 weeks after inoculating, through tip of one leaf, and showing new top growth after brief dormant period and small, ragged, mealy bulb heavily infested,  $\times \frac{1}{2}$ . B. A bulb of Early Yellow Globe from field, showing typical *Fusarium* dry-rot symptoms for comparison with C,  $\times \frac{1}{2}$ . C. A typical *Ditylenchus*-infected, half-grown bulb with outer scale peeled aside to show white, frost-like mealiness. D. A malformed split bulb commonly found in infested areas but not always infected.

of A, B. Buchholz undertook to stamp out the infested areas in these 3 fields. The difficulties incident to steaming seemed insurmountable with the available funds, so sulphur was applied to some of the spots and chloropicrin to one of the largest. The sulphur was broadcast the first week of September

at rates between 1,200 and 3,000 pounds per acre and was then disced in. The chloropicrin was applied through the courtesy and by agents of Innis, Speiden and Company, New York, at approximately 350 lb. per A. in rows and holes 15 in. apart, 8 in. deep, at 4 cu. cm. per hole.

Examinations made in July and again in August, 1939, indicated that applications of sulphur at rates less than 2800 lb. per A., while helpful in reducing infections to a point where onions could perhaps be grown without too much loss, were still not adequate to bring about eradication, at least in one year, according to Chitwood *et al.* (4). On the other hand, where the disease seemed to have been wiped out by the heavier sulphur applications, the onions did not attain full market size. This probably was due to unfavorable pH, which was 4.72 outside and 3.78 inside some of the sulphured areas 10 months after application. The chloropicrin-treated area grew an excellent crop of onions apparently free from nematode injury, conditions having been very favorable with respect to soil moisture and temperature at the time of application.

A survey reported by Newhall *et al.* (14) covering about 1,000 acres in the Canastota area, made in August, 1939, by the writers with the aid of R. L. Clement and I. D. Smith, inspectors of the New York Bureau of Plant Industry, failed to reveal any other infested fields. Less extensive surveys, including 5 acres in the vicinity of Lansing, 125 acres at S. W. Oswego, 7 acres near Fairhaven, 3 acres near Victory, and 125 acres near Savannah, also uncovered no more infestations. But the junior writer and Mr. Clement each found a field between Pine Island and Florida in Orange County, New York, 140 miles (air line) from Canastota, in each of which approximately 6,000 sq. ft. were heavily infested. There is reason to suspect the origin of one of these areas to be the same as Dellaquila's, *i.e.*, set onions originating outside of the State in 1929.

The possibility of the outbreaks recorded here having come from such wild hosts as dandelion, teasel, or strawberry has been considered. Godfrey (6) reported *Ditylenchus dipsaci* on dandelion in western New York and Ontario, Canada, years before. But none could be found in the vicinity of the fields nor was infection secured on dandelion with mass transfers from onion in one trial by the junior writer.

#### SYMPTOMATOLOGY

##### On Seedlings

Onion seed of the variety Ohio Globe was sown in steamed muck to a portion of which was added a suspension of nemas from chopped, infected onions. The soil was kept moist with a mist spray. Emergence of the seedlings was considerably retarded (Fig. 4, C). Stands were reduced from 69 per cent in steamed but noninfested muck down to 47 per cent in the infested muck. Over half of the seedlings that did emerge were diseased. Many of the living seedlings were very pale and assumed thickened, arched, and abnormal shapes by the time they were  $\frac{1}{2}$  in. high. Some of these are shown

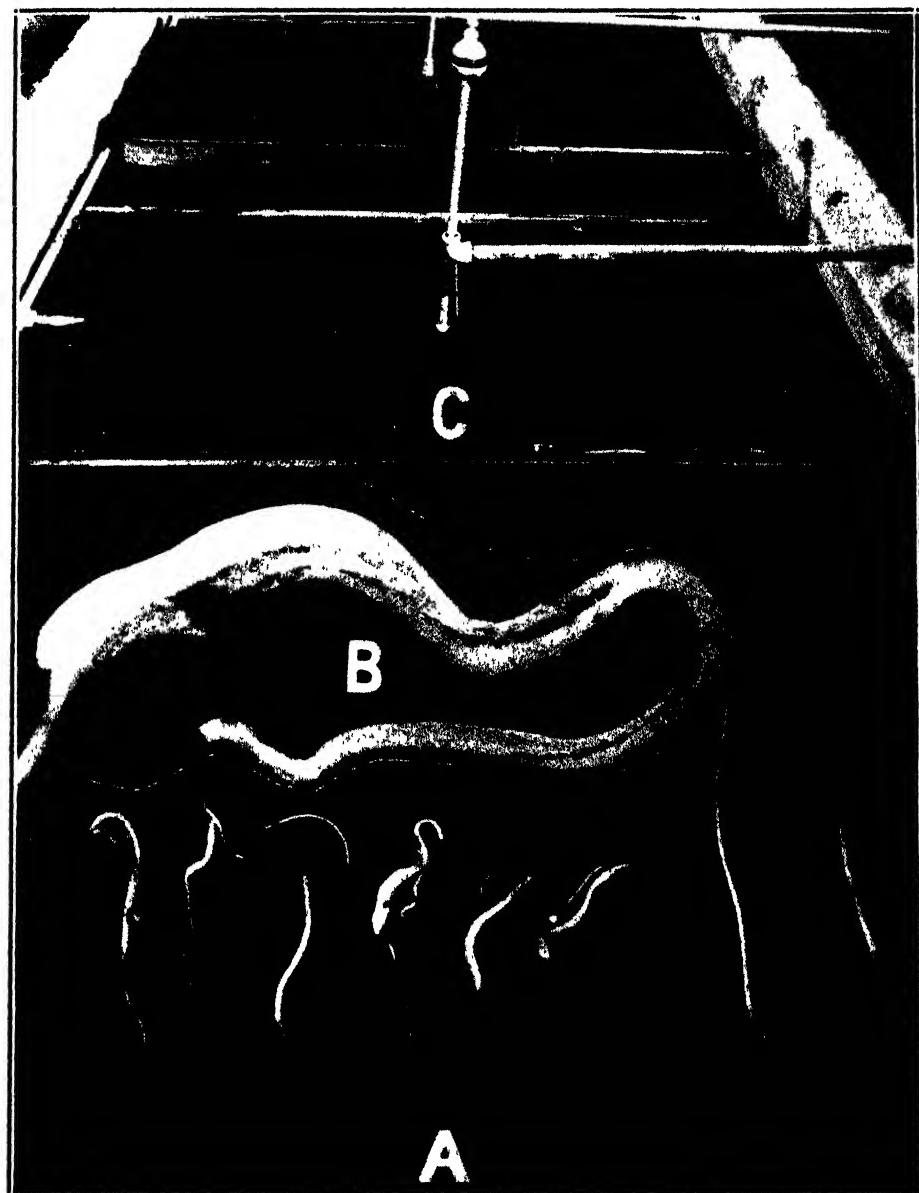


FIG. 4. Seedlings of onions 10 days after sowing in sterilized and reinoculated muck. A. Early symptoms of hypertrophy, distortion, pallor, and cracking on six diseased seedlings compared with 2 normal ones on right, all natural size. B. One of six diseased seedlings,  $\times 5$ ; note puffy, lace-like cracked and twisted condition of entire cotyledon above the stem plate. C. Inoculated bed in foreground where the stand is but 46% of the check and over 50% of the seedlings showing above-ground are diseased. A mist-spray nozzle seen in the center of each bed was used to keep soil moist.

in figure 4, A and B, all in the cotyledon stage. Enlargements somewhat suggestive of those caused by smut, *Urocystis cepulae* Frost, were pronounced, but these are readily distinguished by their color. Splitting of

the epidermis often followed the enlargement. Many seedlings died during the first 3 weeks after sowing, and the survivors sometimes died later. This agrees with growers' observations of the progressive failure of the crop on infested soil. Living nemas could easily be found in the swollen tissues of the seedlings above the stem or root plate but not below it (Fig. 4, B). Over 50 were found in one such seedling 3 weeks after sowing, before the first true leaf emerged.

#### On Older Plants

When onion sets were planted on artificially infested muck in the greenhouse, the first symptoms were observed after 3 weeks and included stunting, light-yellow spotting of the foliage (decolorization), swellings, and open lesions, all of which come under the general heading of "spikkles" or eel-worm spots, as that term is applied by Dutch bulb growers. At this time nemas could be found in leaves and young bulbs, though the latter showed no visible symptoms.

The first field symptoms the writers have seen on onions grown from sets were observed in July as slight stunting coupled with a flaccid condition of many outer leaves. A necrosis or die-back of outer leaf tips and a general weakened condition of the older leaves that prevented them from growing erect was very noticeable (Fig. 2). Within the leaf tissue of diseased plants and, particularly the outer layers of stem tissue, the nema has been found in abundance under the microscope. "Spikkle" symptoms were not seen in the field in 1938 or 1939. Godfrey and Scott (7) found that, on garlic, these symptoms also are lacking.

As the bulb swells to half its final size in July and August, the nemas seem to migrate down from the leaves to the outer scales of the bulbs, where their activity results in a more or less pronounced softening of the parenchyma. At this time a little oblique pressure with the thumb on the upper half of the bulb may result in loosening the outer scale and revealing the soft mealy tissue beneath (Fig. 3, C). This lace-like mealiness looks like thick frost. It becomes more pronounced in the normal course of events as the season progresses and more nematodes develop in the tissues of the rapidly growing bulb. Perhaps ten times as many bulbs showing these symptoms were found in August as in July in the same field plots.

Under the microscope this mealy character is seen to be due to the disappearance of much of the middle lamella in the parenchyma of the bulb scales, leaving the cells in a loose granular pack. The nematodes wander about mostly between the cells, but they have been found within them.

#### Secondary Invaders May Alter Typical Symptoms

Not infrequently examination of suspected bulbs and diagnosis of *Ditylenchus dipsaci* infection is rendered difficult by the presence of so many other invaders. The commonest nematodes found have been *Rhabditis* spp., *Aphelenchus avenae* Bastian, species of *Pristionchus*, *Panagrolaimus subelongatus* (Cobb) Thorne, and *Aphelenchoides parietinus* (Bastian) Steiner.

Of these, *Aphelenchus* and *Aphelenchooides* are the most difficult to distinguish from *Ditylenchus*. Mites (*Rhizoglyphus hyacinthi* Boisduval) and larvae of thrips (*Thrips tabaci* Lindeman) and several maggots may also follow so closely on the heels of *Ditylenchus* as to greatly alter the true picture of a white, mealy, frosty or lacy, dry, non-odororous rot. The presence of these secondary organisms, accompanied as they usually are by many bacteria, results in a moist, malodorous condition and renders the bulb a less congenial place for *Ditylenchus*, which is not a scavenger but an active parasite on living tissue.

#### Infection Experiments

Dry, cured onion bulbs have been artificially infected by the authors with mass transfers of *Ditylenchus dipsaci* from onions and from narcissus. The easiest way to keep this nematode in culture in the laboratory has been by such transfers, the bulb being stored in a cool basement, moisture kept in by wax-paper wrap. After thirty days, the disease may progress to the base of the bulb and occasionally involve every scale.

When a suspension of nemas in a drop of water was placed on 12 sound, uninjured Early Globe bulbs, from which the outer brown scales had been removed, and the onions kept in a moist chamber at room temperature for a month, no signs of infection were observed. But when the skin was previously broken, infection could be detected in two weeks as a soft swelling for half an inch about the point of injury. Laidlow and Price (11) reported that onions transplanted from sterilized to infested soil were not liable to attack unless the bulb is injured in the process or, subsequently, by other agencies.

Red Weathersfield onions growing in the greenhouse were readily infected July 20 by injuring the tips of leaves and injecting a few drops of a water suspension of *Ditylenchus dipsaci*. The resulting bulbs from these plants were small and heavily infected at harvest time 8 weeks later. One plant, which had split to form 3 bulbs, produced 2 normal, healthy ones and 1 rotten bulb of equal size. The nemas had not passed from diseased to healthy bulbs, even though all were attached at the base.

During storage, activity of the nemas continues and bulbs become lighter in weight, somewhat puffy, and more yielding to pressure of the fingers. As deeper layers of the onion become invaded the bulb may swell enough to suggest the term "bloat," which is really only applicable to some bulbs in this storage condition. Secondary rots may obscure this symptom.

#### DISCUSSION

Control of this disease in a region where onions are grown almost continuously is difficult without equipment for steaming between crops. Such equipment is not always readily available. Chemicals have been resorted to in the past 2 seasons, but it is too early to give a clear and authoritative evaluation of them. As already mentioned, the indications are that on muck underlain with marl, but having a pH close to 5.0, 1.5 tons of sulphur

thoroughly disced into the top 6 inches may eradicate the nema. But such heavy applications are likely to reduce the yield of the first crop of set onions considerably. Applications of less than 1 ton failed to eradicate in 3 field trials. Chloropierin, in one field trial at 350 pounds per acre, applied just before a heavy shower, seemed to give complete control, with no crop injury, but is expensive for large-scale eradication. Further tests with these materials were made in 1939 in several fields in Madison and Orange counties (Fig. 1, C, D) and will be reported on later.

Three greenhouse attempts to prove the existence of migrant *Ditylenchus dipsaci* in muck from infested areas by growing a crop of several thousand onion seedlings have all failed. In these cases all onion bulbs that might have harbored the nema were screened out. When infected bulbs previously chopped were mixed with the soil, the resulting infections were numerous (Fig. 4). It must be admitted the soil used in these tests had lain in the field during several weeks of unprecedented, dry, warm weather and had become almost powdery-dry. While not denying the possible persistence of nemas living free in the soil, the writers are inclined to stress diseased bulbs as of far greater importance in the overwintering of this nema. Laidlow and Price (11) reported its survival after 2 years in dried onion plants and after 6 months in dry soil.

In view of the fact that the strain of *Ditylenchus dipsaci* attacking onions has been transferred to rye and the one from rye to onions, there is a question about the wisdom of using either this cereal or oats as a cover crop on infested muck. Since the strain attacking hyacinth and narcissus has been transferred to onions, these crops should be kept a safe distance from soil intended for onions, especially since the disease is known to occur rather commonly on these ornamentals.

The safest crops to use in rotation with onions on infested muckland where maximum returns must be secured would seem to include carrots, lettuce, spinach and beets. Unfortunately, celery and potatoes are listed among the susceptible vegetables.

The length of the rotation that may be needed to completely starve out this pathogen from muckland is the subject of experiments now under way, but some indication may be afforded by the work of others concerned with upland infestations. In England, Hodson and Beaumont (9) have obtained evidence that 3 years is long enough, provided no susceptible weeds are present, such as ribwort plantain, but Walton (23) states that it may persist for 4 or 5 years. Godfrey and Scott (7) report a case in California in which 6 years of continuous lettuce culture apparently starved out the strain attacking garlic.

The results from rotation and fallowing probably depend largely upon the soil moisture and flora. With sufficient moisture the nemas are active, seeking new hosts in consequence of which they use up the foods stored within. Goodey (8) reported laboratory experiments indicating the starva-

tion of this species in moist soil after 12 to 18 months, and the junior writer has noted starvation on agar plates after shorter periods.

It is of more than passing interest that the pest is known to occur in California on garlic and has been found to pass over from this crop to salsify, parsley, and even to celery. Godfrey (6) showed that the strain attacking false dandelion (*Hypochaeris radicata*) is carried within the seed, while he also found evidence indicating that this strain was introduced into the country when that weed was probably brought to our shores many years ago in soil used as ballast by lumber boats. Other cases of seed-borne nemas of this species are well-known. In this connection, Ritzema Bos (18) reported obtaining as high as 3 per cent infection of seed from diseased onion plants. It is, therefore, important as a precaution against further dissemination of this disease that both sets and seeds be grown a good, safe distance from any infested fields.

#### SUMMARY

1. Records of outbreaks of *Ditylenchus dipsaci* occurring on growing onions in North America are confined to New York State, but the likelihood of these all tracing back to commercial sets sold in the State in 1929, but grown elsewhere, indicates that this disease may exist in other places.

2. A description of the disease as it affects onion seedlings and growing bulbs is given. On the former, a stunting, distortion, temporary decolorization and hypertrophy are characteristic. On the latter, a softening of outer scales accompanied by frostlike, odorless, mealiness of the parenchyma tissue is fairly diagnostic.

3. In the absence of rotation, the disease may reduce yields to almost nothing.

4. It has been eradicated once by steam sterilization of one-third of an acre of muckland.

5. The use of sulphur and of chloropierin as more economical soil treatments have shown some promise in preliminary tests.

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## EFFECTS OF SOIL TYPE, SOIL STERILIZATION, AND SOIL REACTION ON BUNT INFECTION AT DIFFERENT INCUBATION TEMPERATURES

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### INTRODUCTION

It is known that the degree of infection of a susceptible variety of wheat with *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn is dependent upon a number of environmental factors. Experimental data indicate clearly that soil type (3, 8, 9, 13), soil temperature (3, 5, 7, 8, 9, 10, 14), and soil moisture (2, 3, 7, 8, 9, 14) are all involved and any one of these factors may limit infection. Less is known of the effect of post-infection environmental factors. Faris (3) found no evidence that the growth of the host, after the germination stages, had marked effect on the development of bunt in the winter varieties Dawson and O.A.C. No. 104. Smith (12), however, has shown that high temperature during this period limits the development of smut in Hope wheat but not in Jenkin. Variations in these environmental factors as they occur in different wheat-growing areas, affecting both the host and pathogen, undoubtedly account for differences in the response of wheat varieties to any one race of *T. tritici* or *T. levis* (11). As part of a comprehensive study of

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this problem, tests were made at the Arlington Experiment Farm, Arlington, Virginia, involving the effect of soil type, soil sterilization, soil reaction, seed size, and different temperatures during the seedling growth stage.

#### METHODS AND EXPERIMENTAL RESULTS

##### Soil Type

In studies on the effect of soil type as a factor influencing infection of wheat by *Tilletia tritici* and *T. levis*, comparisons for the most part have been made between the effects of light sandy soils, presumably low in organic matter, and heavier soils higher in organic content. Results obtained by Faris (3), Volk (13), Rabien (9), Leukel (8), and others indicate that the latter types are more favorable for bunt infection than are the former. In the present experiment, two productive soils were used. One obtained from University Farm, St. Paul, Minnesota, was mapped in the Ramsey County, Minnesota, soil survey (1914) as Hempstead silt loam. It was a surface soil of a dark silt loam high in organic matter with a pH value of 6.7. The second type was the surface soil of a Mendon loam obtained from the Experiment Station Farm at Logan, Utah. This was a grayish-brown, friable silt loam, free from harmful accumulation of salts or alkali, containing little or no free lime and with a pH value of 8.1. These soils were adjusted to approximately 50 per cent of their moisture-holding capacity with sterile distilled water and placed in galvanized-iron germination pans, 2 inches deep, 4 wide and 8 long. Seed lots of Marquis (C.I.<sup>2</sup> 3641) and Thatcher (C.I. 10003) wheat were each separated into large and small seed by using a No. 8 Tyler screen (8 meshes to the inch). The kernels that did not pass through this screen were considered large and those that did were considered small. These lots of large and small kernels were inoculated with chlamydospores of *T. levis*, race L-2 (11), at the rate of 0.5 grams of chlamydospores to 100 grams of seed. Three replications of 200 seeds each were planted at 1 inch depth in the soils, placed in incubation chambers at 5°, 10° and 15° C., and left there until the seedlings emerged from the soil to a height of approximately 1 inch. The seedlings were then transplanted to greenhouse beds, where all were subjected to the same post-infection environmental conditions until maturity. Thus, under the conditions of these experiments, differences in percentages of smut at maturity should be due to environmental influences effective only during the incubation periods. The resulting data are given in table 1, and those giving the responses of the Marquis wheat grown from large seed are shown graphically in figure 1.

From the above data it is clear, as pointed out by previous workers, that soil type may influence the degree of infection of wheat with *Tilletia levis*. Apparently the differences in effect that have been observed between the light sandy-type soils and those higher in organic matter hold also for 2 productive soils used in these tests, both of which are high in organic matter.<sup>3</sup> The

<sup>2</sup> C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

<sup>3</sup> Organic matter expressed in percentage total nitrogen was found to be 0.264 for

TABLE 1.—*Percentages of bunt in Marquis and Thatcher wheat grown from large and small seed inoculated with *Tilletia levis*, L.-<sup>o</sup>, and germinated at different temperatures in Mendon loam and Hempstead silt loam, nonsterilized and sterilized, Arlington Experiment Farm, Arlington, Va., 1935-36*

Incubation temperature °C.	Soil type	Size of seed	Marquis	Percentage of heads smutted											
				Non-sterilized soil				Sterilized soil				Non-sterilized soil			
				Series			Av.	Series			Av.	Series			Av.
5	Mendon	Large	71.0	47.5	73.3	63.9	59.3	40.0	56.5	51.9	78.6	54.5	84.3	72.5	43.5
			20.8	40.5	31.4	30.9	94.6	90.2	98.0	94.6	28.3	29.6	32.9	30.3	98.9
10	Mendon	Small	79.5	73.4	84.2	79.0	40.0	48.1	57.0	48.4	90.5	70.7	88.8	83.3	32.2
			37.8	43.5	40.3	40.5	98.7	100.0	100.0	99.6	29.2	67.5	47.5	48.1	93.6
15	Mendon	Large	95.1	93.2	94.4	94.2	71.0	82.8	81.6	78.5	92.6	95.1	88.6	92.1	29.1
			82.6	75.8	87.0	81.8	79.3	93.2	81.0	84.5	66.3	77.1	67.3	70.2	86.0
	Hempstead	Small	89.7	99.1	95.4	94.7	77.2	83.8	93.3	84.8	91.6	89.8	82.3	87.9	55.7
			74.2	84.1	85.9	81.4	76.3	89.3	87.8	84.5	76.5	70.5	80.8	75.9	88.5
	Mendon	Hempstead	73.8	93.3	86.0	81.0	56.6	50.7	67.5	58.3	75.2	76.7	87.7	79.9	50.8
			84.3	73.9	74.2	77.5	24.6	23.9	19.2	22.6	59.4	68.1	75.6	67.7	6.0

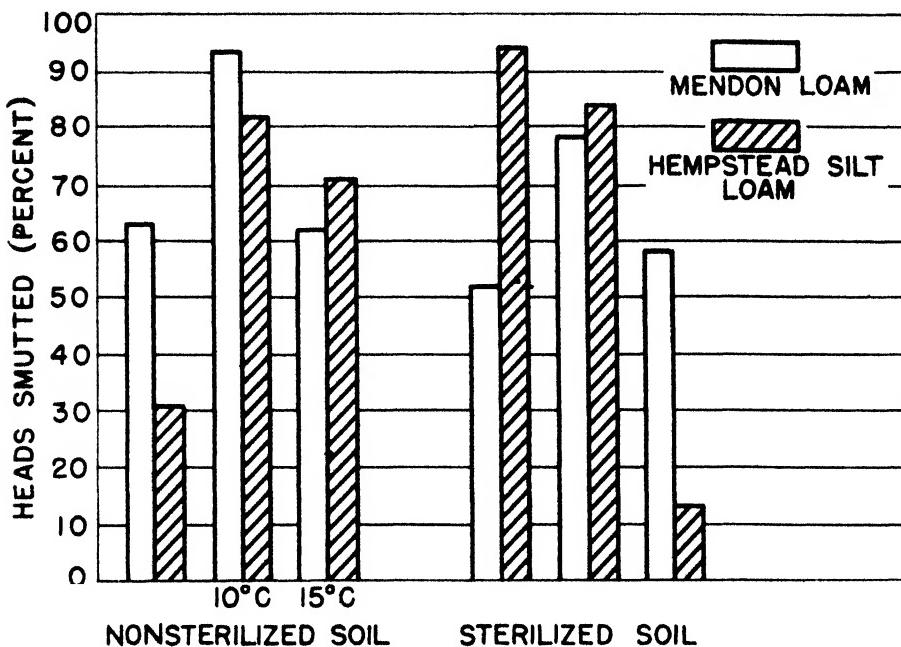


FIG. 1. Percentages of infection in Marquis wheat, large seed, germinated in non-sterilized and sterilized Mendon and Hempstead silt loams.

present data indicate, however, that the differences in effect in the 2 soils are contingent upon the temperatures prevailing during the incubation period. At incubation temperatures of 10° and 15° C., there were no marked differences in the effect of the 2 nonsterilized soils on the degree of infection of Marquis wheat. At 5° C., however, there were marked differences. When the inoculated large seed was germinated in the Mendon loam there was 63.9 per cent smut as compared with only 30.9 per cent in the Hempstead silt loam. In comparable tests with Thatcher, higher percentages of smut developed where the inoculated large seed was germinated in the former than in the latter soil at all 3 incubation temperatures. However, as with Marquis, the greatest differences were at 5° C., 72.5 per cent developing in the Mendon loam as compared with 30.3 per cent in the Hempstead silt loam.

In general, less vigorous wheat seedlings develop from small seed than from large. Since, in some years, only small and shrunken seed of a variety is available, size of seed was made another variable in these experiments. Heald (4) and Holton and Heald (6) have found that percentages of bunt infection may vary, depending upon the origin of the seed. In order not to introduce this variable, the lots of large and small seed used were of the same origin.

Bayles (1) found a tendency for the seedlings from smaller seed of Marquis to be more susceptible to bunt than those from the large. In the Mendon loam and 0.196 for the Hempstead silt loam. The writers wish to express their appreciation to Mr. P. R. Dawson of the Division of Soil Fertility, B.P.I., for these analytical data.

results obtained by the writers with Marquis and Thatcher differences in percentages of infection in the large and small seed were negligible with an incubation temperature of 10° C. On the other hand, at 5° C. and 15° C., there were slight, though consistently higher, percentages of bunt in the plants from the small seed. The results in this experiment also emphasize the fact that any differences in the effect of the two soils were contingent upon temperatures prevailing during the incubation period.

### Soil Sterilization

In certain wheat-growing areas, infection from soil-borne chlamydospores of *Tilletia* spp. is common. It is, therefore, necessary to sterilize such soils for use in controlled, greenhouse experiments involving pathogenicity tests with individual races. The question arises whether pathogenicity tests in sterilized soils are comparable with those made in nonsterilized soils. The sterilization was accomplished by subjecting portions of each soil before planting to steam at 15 lb. pressure for 4 hours on one day and 2 hours on the next.

Data on the response of the two varieties (Marquis and Thatcher) to *Tilletia levis*, race L-2, when incubated in the sterilized and nonsterilized soils, are recorded in table 1, and the data on the response of Marquis are shown graphically in figure 1.

With Mendon loam, steam sterilization effected a reduction in percentages of infection in Marquis and to a somewhat more marked degree in Thatcher at each of the 3 incubation temperatures. With the Hempstead silt loam entirely different results were obtained. In this soil, steam sterilization effected marked increases in infection in both varieties when incubation temperatures were at 5° C., little or no effect at 10° C., and very marked reduction at 15° C. Thus, for example, for Marquis wheat grown from large seed, the percentages of infection at 5° C. was only 30.9 per cent infection in the nonsterilized soil as compared with 94.6 in the sterilized soil. At 15° C., the reverse effect was obtained with 71.7 per cent infection in the nonsterilized soil and only 13.7 in the sterilized. It is also significant that in the sterilized Hempstead loam the optimum temperature for infection was shifted from 10° C. to 5° C.

A comparison in infection response also may be made between the Mendon and Hempsted soils when nonsterilized and sterilized. When the two soils were not sterilized, the differences in effect on bunt infection were pronounced only at 5° C., incubation temperature. At 5° C., higher percentages of infection developed in the Mendon and Hempstead soils. However, when the soils were sterilized, the comparative effect of soil type is entirely different at both 5° and 15° C. At the former incubation temperature, higher percentages of bunt developed in the Hempstead than in the Mendon soil. On the other hand, at 15° C., the reverse was true with higher percentages of infection in the Mendon than in the Hempstead. Unfortunately, there was not sufficient soil in the original shipment to repeat these experiments in a second season. However, the similarity of results obtained with the two

varieties and with large and small seed of both varieties gives emphasis to the conclusions that are drawn from these tests made in a single year.

#### Soil Reaction

There are wide differences in the pH values of soils in the same and different sections of the country where bunt tests are being made and the question arises as to the extent to which soil reaction may affect infection by the bunt organisms. Available data are somewhat confusing. Rabien (9) reported that pH 5.0 represents the acid limit for germination in soil of chlamydospores of *Tilletia tritici*. Leukel (8) obtained only 5.8 per cent smut in Purplestraw in soil with a reaction pH 5.6. However, there is evidence that in certain soil types approximately as high percentages of infection may be obtained under conditions of high acidity as of low. The writers obtained 73.5 per cent infection in Marquis wheat grown in an eroded Chester loam from Fairfax County, Virginia, with pH 4.8. Furthermore, when the pathogenicity of 9 physiologic races of *T. tritici* was tested on the spring wheat variety Ulka (C.I. 11478) at the Arlington Farm, and at Logan, Utah, in soil of pH values of 5.4 and 8.1, respectively, the lowest percentages of infection obtained with any of the 9 races were 85.5 at the former station and 91.9 at the latter. These differences are considered nonsignificant.

Because of these apparent inconsistencies, tests were made under controlled greenhouse conditions at Arlington Farm with different soil types and different incubation temperatures. Two soils were used. One, obtained near Annandale, Va., was mapped in the Fairfax County, Va., soil survey (1915) as Chester loam. The sample was an eroded type with a pH of 4.8. The second was a surface soil obtained from the Arlington Experiment Farm. It had a pH of 4.8 and was mapped in the same soil survey as Keyport silt loam. To each of these, different amounts of calcium carbonate were added to obtain a range of pH up to 8.1, as indicated in figure 2. All soil reactions were determined by means of a Beckman pH meter. After the addition of calcium carbonate, the soils were adjusted with tap water to 50 per cent of their moisture-holding capacity. Inoculated seed of Marquis wheat was sown in the soil pans, as previously described, and incubated at temperatures of 5°, 10°, and 15° C. There were 4 replications for each soil and temperature condition. When the seedlings emerged approximately an inch, 45 were selected at random from each pan and transplanted to greenhouse beds, the soil of which had a pH value of 5.8. Percentages of infection were obtained on the basis of culm counts. However, under the conditions of these experiments, there was little or no tillering; so, percentages of infection approximate the plant counts. The data are recorded graphically in figure 2.

In both Chester and Keyport soils the percentages of smut increased as the soil acidity decreased over the approximate pH range of 4.8 to 7.0. In no case did an increase in the pH value above 7.39 produce any further increase in smut. The greatest increase in smut with decreasing soil acidity occurred in the eroded Chester loam between pH 4.8 and pH 5.29. These increases

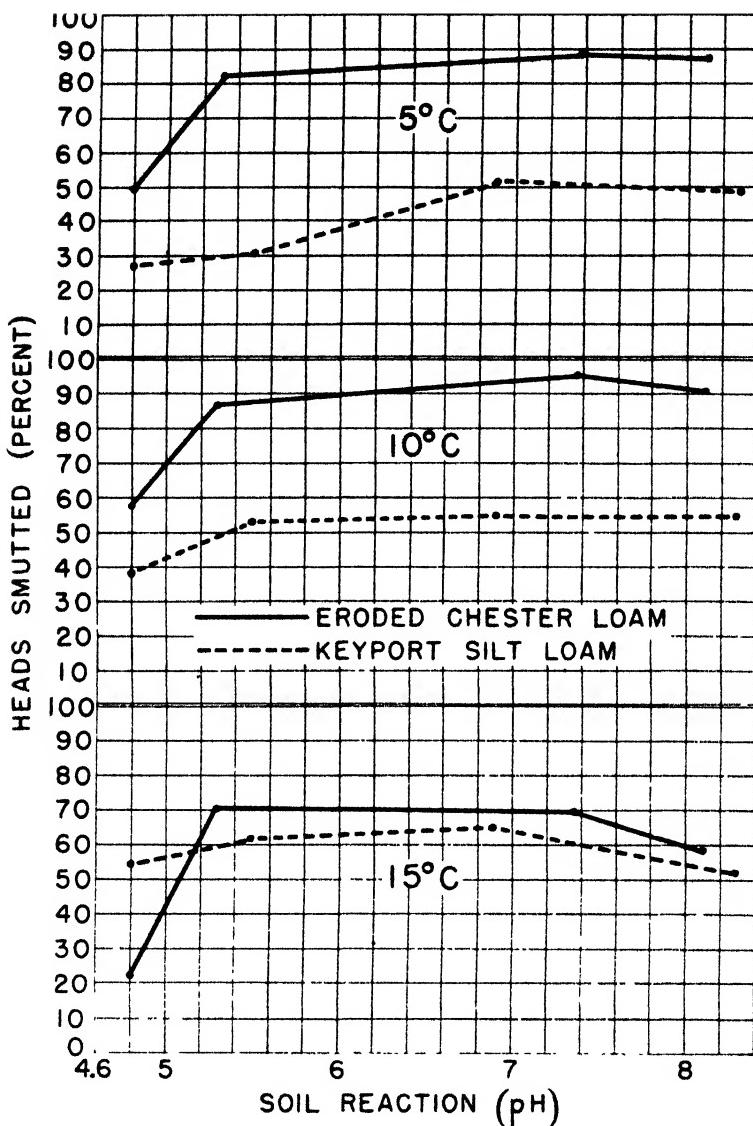


FIG. 2. Effect of soil reaction on bunt infection in Marquis wheat.

occurred at all 3 incubation temperatures. At certain incubation temperatures, however, soil type is a more important factor than the pH value. Uniformly higher percentages of bunt were obtained in the plants from seedlings grown in the eroded Chester loam than in the Keyport silt loam at each reaction tested when the incubation temperatures were 5° and 10° C. At 5° C., the average bunt infection for all pH values in the Chester loam was 76.7 as compared to 39.3 in the Keyport loam. At 10° C., the corresponding percentages were 82.6 and 50.5. When incubation temperatures were maintained at 15° C., differences in the two soils were minimized at all pH values

except 4.8. It will be noted that at pH 4.8 there was 34.7 per cent more smut in the Keyport loam than in the Chester loam, which results are opposite from those obtained at the 5° and 10° C. incubation temperatures.

#### SUMMARY AND CONCLUSIONS

It is apparent from results obtained that bunt infection in a susceptible wheat variety is affected by soil type. Furthermore, the degree to which it is affected depends on the soil temperature during the period in which infection may take place. For example, when both Marquis and Thatcher seedlings from large seeds were incubated at 10° and 15° C., there were no appreciable differences in the amount of infection in the Hempstead and Mendon loams. At 5° C., however, Marquis developed 33 and Thatcher 42.2 per cent more smut in Mendon than in the Hempstead loam. Similar conclusions may be drawn from an experiment in which a comparison was made between the effect of the eroded Chester loam and Keyport clay loam. At 5° and 10° incubation temperatures, consistently higher percentages of bunt developed in the former than in the latter soil type. At 15°, however, these differences were less, except when the soils were highly acid; then, 34.7 per cent more smut developed in the Keyport than in the Chester loam. Thus, soil type and temperature during the incubation period are closely interrelated in affecting the degree to which a variety may be infected. It has not been determined whether the results obtained are due to a modification of the resistance of the host plant or to the direct effect on the pathogen.

Generalizations may not be made as regards the effect of soil sterilization on bunt infection. Sterilization of the Mendon loam effected reductions in bunt infection in Marquis and Thatcher grown from both large and small seed at all 3 incubation temperatures. Reductions were, in general, greater in Thatcher than in Marquis. With Hempstead loam, on the other hand, the effects of soil sterilization were irregular. At the 5° incubation temperature, instead of a reduction, as occurred in the Mendon soil, sterilization of the Hempstead soil effected an increase in infection from 30.9 per cent in the nonsterilized to 94.6 in the sterilized soil. On the other hand, at 15° incubation temperature, sterilization caused a reduction in infection from 71.7 to 13.7 per cent. The effect of soil sterilization was, in general, less pronounced at the 10° incubation temperature. It may be concluded that pathogenicity tests made in steam-sterilized soils of the Hempstead and Mendon types are not comparable with those made in either of the two soils when not sterilized.

The present experiments on the effect of soil reaction on bunt infection are not extensive enough to answer all of the questions of inconsistency in previous experiments. The data indicate, however, that change in pH may effect a change in the degree of bunt infection in Marquis wheat when germinated and incubated in either the Chester loam or the Keyport clay loam. In both types of soil with an initial pH of 4.8 there was, in general an increase in percentages of infection from the points of high acidity to

a point approaching neutrality, the most marked effect being in the change from pH 4.8 to approximately 5.5. Evidently the degree to which pH affects bunt infection varies with the soil type and incubation temperatures. For example, at 5° and 10° temperatures and at each reaction tested, higher percentages of bunt were obtained in the Chester loam than in the Keyport clay loam; and it is apparent that at these temperatures, soil type affects bunt infection to a greater degree than does change in pH. However, at the 15° incubation, differences due to soil type are less and the soil reaction becomes the more important factor, for at pH 4.8, 34.7 per cent more smut developed in the Keyport than in the Chester loam.

In these experiments there was a trend toward higher percentages of bunt infection when small seed was used in comparison with the large. The seed-size factor may, therefore, be considered a minor factor in effecting variability in response of a variety to bunt. Soil type and certain incubation temperatures were major factors, and the present experiments emphasize the importance of the interrelation of the two factors. Variation in their interrelation may account for seasonal differences in response of a variety to a race of the smut fungus and for inconsistencies in the reaction of a variety to a race of bunt when tested at different places.

It should be noted that in these experiments the effect of the environmental factors referred to has been studied with relation to a single race of *Tilletia laevis*, namely, L-2.

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# ANTHRACNOSE AND CLADOSPORIUM STEM SPOT OF PEONY

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The identity of the peony stem spot, described as anthracnose by Whetzel (14) in 1915, has long remained obscure, and specimens have been rarely collected or reported. In routine examinations of peony specimens through a period of over 10 years, the writer has encountered what seems to be this disease but once, on material collected by J. B. Demaree at Willard, North Carolina, August 30, 1938. Anthracnose is not included among the peony diseases observed in Quebec by Coulson (1), nor is it mentioned in the compendiums of diseases of ornamental plants published by Tilford (12) for Ohio, and by White (15) and Pirone (9) for New Jersey. No reference to it is found in the books on diseases and pests of ornamental plants in Europe by Flachs (3) and Pape (8). Although Martin (5) suggested that a red spotting or generalized stippling of stems, leaves, and flowers of *Paeonia*, from which he isolated *Cladosporium paeoniae* together with a budding fungus, was identical with Whetzel's peony anthracnose, a comparison of the descriptions and illustrations of the two diseases shows rather marked differences. The writer (13) suggested the name "measles" for the peony disease, characterized by numerous small red or purple spots on all aerial parts, to which Martin referred. Gregory and Davis (4) described a leaf and stem spot of unknown cause affecting peonies in Indiana, which appears to be of the same type, although one cannot be certain from their illustration.

The North Carolina specimen of anthracnose (consisting of stems only) bore immature acervuli or pyrenidia beneath the epidermis in the ash-gray, depressed center of the lesions, in which small numbers of sub-hyaline or slightly greenish, nonseptate spores of elliptical form, measuring  $4-6 \times 2-2.5 \mu$ . were borne. Other parts of the stem, where the entire epidermis had turned gray, bore dark-brown extruded masses of similar spores. The fungus was provisionally designated as a *Leptothyrium*, but these specimens were inadequate for precise identification or for isolation of the pathogen. However, the correspondence with Whetzel's description and figures was very close.

## SYMPTOMS

### Red spot or "measles"

Although anthracnose has been rarely observed, the red stem spot, or "measles" type of infection has occurred widely though somewhat infrequently. Strikingly conspicuous examples of this malady were received by the writer from a commercial peony grower of Onargo, Illinois, on June 1, 1924. Martin referred to its prevalence (in the vicinity of Washington, D. C.) in 1929. It occurred here commonly again in 1932. J. R. Kienholz reported this disease to the writer from Oregon in 1932, and A. L. Pierstorff sent specimens from Ohio in 1934. In each instance the plants were promi-

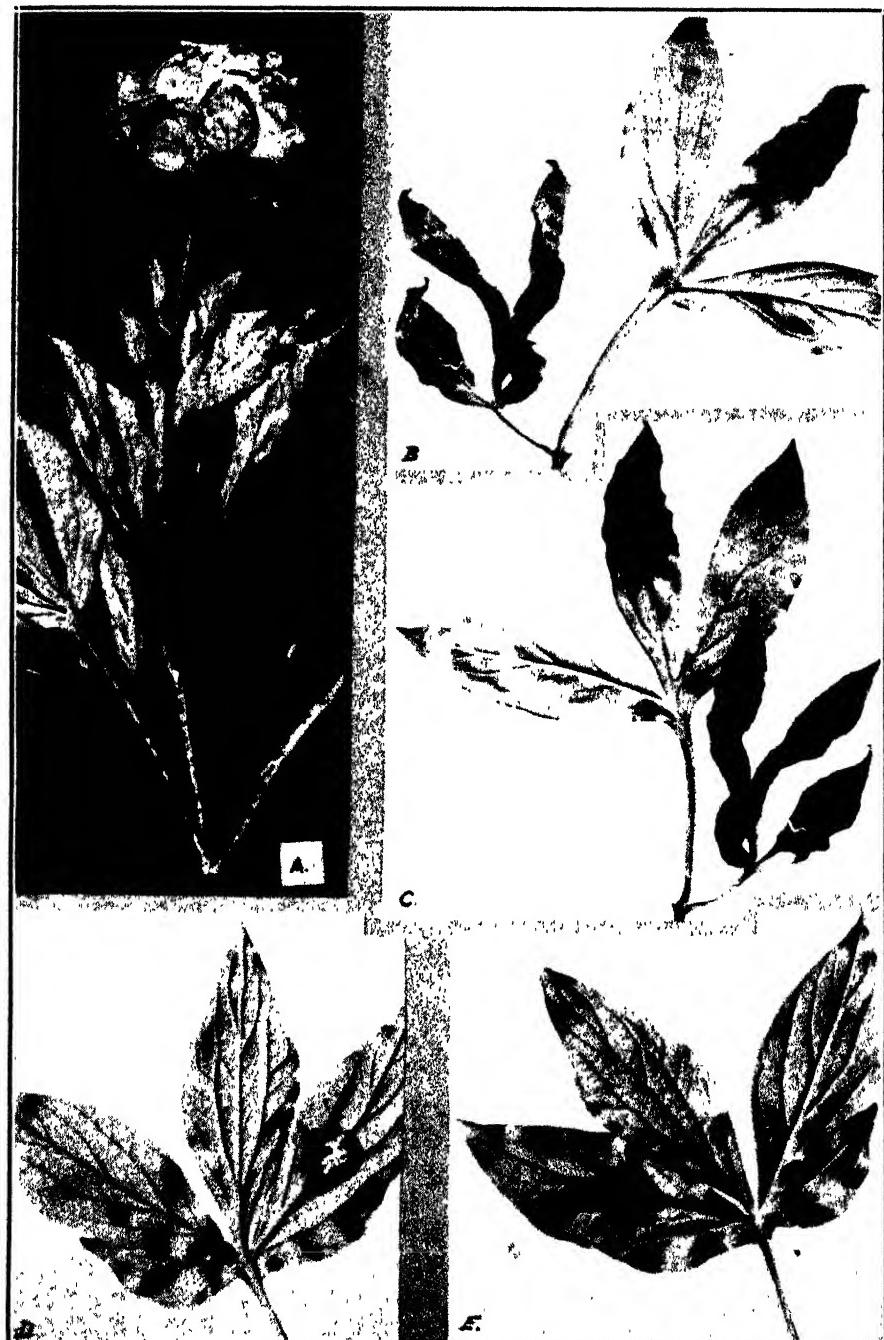


FIG. 1. Spotting of stems, leaves, and flower of peony caused by *Cladosporium paeoniae*. B-C. Lesions on peony leaves resulting from artificial inoculation with a budding fungus isolated from stem and leaf spots; the distinct center and zonate margin are characteristic; B, lower surface; C, upper. D-E. Lesions on peony leaves resulting from artificial inoculation with the conidial stage of *Perizella lythri*; D, lower surface, E, upper.

nently disfigured by or before the period of full bloom. In June, 1938, a commercial grower of peonies at Concordville, Pennsylvania, submitted specimens of the variety Avalanche from a field which had been severely damaged for flower cutting by this disease.

Since the red stem spot appeared to be increasing in importance a study of it was begun to determine its cause and to clarify its relation, if any, to anthraenose. The discontinuance of this study before its completion makes advisable the publication of the results to date.

Stems, leaf stalks, and blades, floral bracts and petals are susceptible. Sometimes there appears to be a gradient of infection diminishing from near the ground level to the apical parts, or the upper leaves and flower stalk also may be densely spotted. The stem spots are definitely raised, elliptical to elongate, usually discrete but sometimes confluent into streaks, typically about 2-4 mm. long and 1-2 mm. wide. They are very similar to the stem lesions caused by *Cladosporium paeoniae* Pass. as described by Meuli (6). The leaf spots are much smaller, some being mere flecks, less than 1 mm. wide. They are most prominent on the dorsal surface, but the larger spots are visible on the upper surface also. There is a predilection for the veinal tissue, and the spots on the principal veins are larger and more elongate than those on the interveinal portions. The calyx-like floral bracts are attacked similarly to the leaves, and the petals also may bear, sometimes very numerously, small reddish spots of the same pattern. The stem spots increase in size but little after they first become visible, although in mature stems there is a slow extension with loss of the definite margin. Even on mature stems they are essentially superficial, sometimes only one or two cell layers under the epidermis showing disorganization and never the wood. Both leaf and stem spots are typically a purplish or brownish red throughout, usually without differentiation into a lighter center and darker margin; although in age the center may become brown, black, or gray and is cracked and depressed. The reddish color persists in the infected area, even in stems that have died and turned brown. Ordinarily the infected stems, even when heavily spotted, remain alive until the natural end of the growing period.

Sections of the lesions made at an early stage of development show a rather sparse, predominantly intercellular mycelium present in small groups of epidermal and hypodermal cells. The smallest lesion may involve no more than six to ten cells. The contents of the invaded cells coagulate to a brown gummy mass, sometimes with deposition of a purple pigment, and the walls become thin, eroded, and incoherent. There is no association between the lesions and the few stomata present on stems; the leaf stomata are hypophyllous and the lesions may or may not surround them; but, since many of the leaf lesions originate on the veins, infection is clearly not dependent on stomatal penetration. In some sections the internal mycelium bore apically enlarged branches suggestive of the chlamydospores or hyphal knots, and also of the aerial conidia, produced by *Cladosporium* in culture.

The intercellular hyphae were conspicuously intertwined. The surface of old lesions also may bear sparsely the characteristic mycelium and spores of *Cladosporium*. Some of the stem lesions at an older stage become definitely rounded and protuberant, as if in response to the formation of a fungus fruit body within; but, aside from the superficial growth of *Cladosporium*, and sometimes of *Alternaria*, they do not develop definite fruit bodies before winter.

#### Etiology

Examination of the infected planting at Concordville, in July, showed that not only the red spot was prevalent on stems and leaves but that there was a severe infection of leaf blotch (*Cladosporium paconiae*), and also a small circular leaf spot having a light-brown center and a dark-brown, or purple, distinct margin. Some of the stems had turned a dark-brown from the ground line to the first or second leaf, but the red-spot lesions could still be distinguished in the general cortical necrosis. Salmon-pink sporodochia were found on some of the dead stems.

Isolations made at this time yielded *Cladosporium paconiae*, *C. herbarum*, *Alternaria*, and a *Glocosporium* that appeared identical with a conidial culture of *Glomerella cingulata* Stonem., obtained from *Rubus*. Stems showing different stages of the disease were collected in July and October for storage under various conditions. Arrangements were made with the grower to carry out experiments on the importance of infected stems as a source of the disease in a hitherto uninfested part of the field, and on the effect of removal of stems at various times, from fall to late winter, on the recurrence of the disease in the infested part of the planting.

Examination of the stored stems kept outdoors at this Station, or in a 10° C. chamber, showed in late winter the development of brown pyrenidia bearing cylindrical or slightly curved, continuous, hyaline spores. Pure cultures were readily obtained, and the fungus was identified by J. A. Stevenson and Edith Cash as *Pezizella lythri* (Desm.) Shear and Dodge, the pyrenidial stage of which is *Sclerotiopsis concava* (Desm.) Shear and Dodge. These colleagues also pointed out that *S. testudinea* Dearness, originally described (2) on dead stems of peony, might be the same fungus, as both concave and convex pyrenidia occurred on these stems; the latter is therefore probably to be regarded as a synonym of *S. concava*. It might be noted that further nomenclatorial changes applicable to this fungus have been proposed (7), viz., *Discohainesia oenotherae* (Cke. and Ell.) Nannf. for the ascigerous stage, and *Hainesia lythri* (Desm.) v. Höhn. and *Pilidium concavum* (Desm.) v. Höhn. for the two conidial stages, but their discussion need not be entered into here.

The same fungus also was found on dead peony stems in the Station plot, and on stems from the field at Concordville, which were collected periodically during the spring. Moreover this fungus was isolated by tissue cultures from the first lesions of red spot that appeared on stems of the current year's growth at Concordville, on May 1, 1939. Several collections of *Sclerotiopsis*

on dead stems of peonies imported from Japan have been made by the United States plant quarantine stations at Seattle and San Francisco.

Tests of *Sclerotiopsis concava* for pathogenicity to peony showed that it is definitely parasitic. However, the characteristic lesions of red spot, especially on stems, were not reproduced by artificial inoculation. The degree of succulence appears to be an important factor in its pathogenicity. Inoculation, without wounding, of the youngest available but still fairly mature stems gave negative results. On petioles and blades of young leaves inoculated with a spore suspension without wounding, a diffuse necrosis developed, causing, under moist-chamber conditions, collapse of the stem and the production of large, light-brown, zonate spots on the leaves. Sporodochia corresponding to *Hainesia lythri* were produced copiously on the leaf spots. The fungus was readily reisolated from tissue plantings and from spores. Subsequent tests on mature peony leaves showed but slight pathogenicity, small (1-2 mm.) brown, somewhat angular spots being produced without the development of fruiting bodies. Comparison of these lesions with those previously mentioned as associated with *Cladosporium* leaf spot in the field, suggests that *Sclerotiopsis* probably occurs as a natural parasite on peony leaves. A collection of peony leaf spot submitted to the writer from Suitland, Maryland, in 1934 appears to be of this type but lacks definite fruiting bodies of the *Hainesia* or *Sclerotiopsis* types; it differs distinctly from *Botrytis* or *Cladosporium* leaf spots. The lesions produced by *Sclerotiopsis* on peony foliage are of a wood-brown to fuscous color on both surfaces as contrasted with the chestnut-brown below and taupe-brown to blackish-brown above of *Cladosporium* spot, and are further distinguished from *Cladosporium* spots by the production, at maturity, of the conidial stage *Hainesia*, or the pyenidial stage *Sclerotiopsis*. Although only a few inoculations at controlled temperatures were made, infections occurred only in the range 14° to 22° C., and the lesions developed most rapidly at 18° to 22° C.

The occurrence of this fungus on some 50 different hosts has been reported by Shear and Dodge (11), who also showed by experimental inoculations that it is parasitic on leaves and canes of various *Rubus* spp., on other woody plants, as *Rhus*, *Prunus* and *Salix*, and on several herbaceous plants, especially of the Onagraceae. Its association with weeds, therefore, becomes a matter of importance in its control in peony plantings.

Besides pyenidia of *Sclerotiopsis*, the overwintered stems bore very numerous minute black, superficial or subcuticular sclerotium-like bodies of a different fungus, associated with which there were many rod-shape, hyaline, nonseptate spores averaging  $4-6 \times 1-1.5 \mu$ . There was also a thin dispersion of mycelium and conidia of *Cladosporium*, cultures of which resembled *C. herbarum* more than *C. paeoniae*.

When stems bearing only the pyenidia of *Sclerotiopsis* were placed among the developing shoots of a potted peony and were thoroughly syringed with water several times, the new shoots developed in 10 to 12 days large,

brown, sunken lesions. When stems bearing the small black sclerotia, together with *Cladosporium*, were similarly used as inoculum, the stems and leaves of the inoculated plant developed in about a week an extremely dense infection of typical red spot or "measles." Reisolations from these spots, on stems or leaves, yielded both *Cladosporium* and a hyphomycete that produced (on corn-meal agar) a submerged or appressed mycelium bearing numerous globular masses of spores pleurogenously or on short lateral branches that were sometimes rameose with slightly inflated sterigmata, but in the main unbranched and bearing terminally a loose cluster of spores. The latter were similar in form and size to those associated with the sclerotia on overwintered stems, though somewhat more variable, some being rod-shape and about  $3 \times 1.5 \mu$ , others were elliptical in section and ranged from  $4 \times 2$  to  $7 \times 2 \mu$ . This fungus also was obtained repeatedly from tissue plantings of natural red-spot infections, usually in association with *Cladosporium*. It is doubtless the same fungus in the "budding phase" that Martin (5) isolated from red-spot infected peonies. Martin also refers to a "fumagoid phase" and states that *Cladosporium paeoniae* developed in cultures of the budding fungus. In the writer's cultures also, a fumagoid phase developed with age. Thick-walled, olivaceous to fuscous cells of globose to ovoid form, 8 to  $12 \mu$  in diameter, developed in simple or sparingly branched chains, or sometimes in irregular aggregates. The different types of aggregates consisted of about 5 up to 50 cells; the larger ones were sclerotiod and were barely visible under a hand lens. In old cultures they imparted a fuscous to black coloration to the entire stroma. No further development was observed in these cultures.

Although the "budding fungus" was often associated with *Cladosporium paeoniae* in isolation plates, it was readily separated by dilution-plating and it, together with its fumagoid phase, appears to be a distinct entity rather than a developmental phase of *Cladosporium*. In the initial stages of its development from a germinating spore in water or on agar, the fungus resembles *Cephalosporium*, but the appressed and viscid character of the mature thallus differs widely from typical *Cephalosporium* species. The fumagoid phase also serves to distinguish it. The form designations *Pseudosaccharomyces* and *Pseudofumago*, as used by Martin, will serve adequately to characterize it until more information about its life cycle is available.

This fungus also is pathogenic to the peony, causing an extensive, dark-brown, moist necrosis when inoculated into wounded stems, and light-brown, dry spots on leaves with or without wounding. The leaf spots resemble those resulting from inoculation with *Pezizella lythri*, except that no pycnidia or sporodochia are produced, but mycelium and spores typical of the budding phase in agar cultures develop copiously on the surface. Experimental inoculations were successful within the range  $10^{\circ}$  to  $27.5^{\circ}$  C., but the growth of the lesions was most active at  $18^{\circ}$  to  $22^{\circ}$  C.

Reference has been made to the frequent association of one or more types of *Cladosporium* with peony stem spots. Meuli (6) showed that *C. paeoniae*

caused stem lesions of the red spot type, as well as the characteristic dendriform leaf spots. In about 200 isolations made by the writer from red spot on stems and leaves, using a 10 per cent Chlorox (5.25 per cent sodium hypochlorite) wash and planting the tissue pieces, without rinsing, on corn-meal agar, *C. paoniae* was obtained alone in the majority of instances, or associated with the budding fungus. Similar results have been reported by others. In view of the infrequent association of other organisms with *Cladosporium* in lesions of this type, and their inability, even when pathogenic to peonies, to reproduce the characteristic spotting, there is strong circumstantial evidence for regarding *C. paoniae* as the sole pathogen. The principal questions remaining are what innate or environmental factors determine the small, discrete type of stem and leaf infection as contrasted with the characteristic large leaf blotches and what factors bring about the early-season, pathogenic activity of an organism that has been regarded as able to invade chiefly mature and moribund tissues.

The answer to the first question may be the relatively low virulence of *Cladosporium paoniae*; that is, the lesions are small and circumscribed when the host tissues are young and vital, whereas infections that are initiated in mature tissues show the characteristic rapid enlargement. Some of the early static lesions also become active as the tissues mature. The relative virulence of the pathogen in this case bears an almost inverse relation to its importance as a disease-producing organism, as it is the profusion of small spots on the stems, foliage, and flowers of the peony during the period of its ornamental value that is economically important. The mature leaf-spot phase is usually of little consequence, except as it creates a reservoir of contamination, as there is ordinarily no serious contraction of the vegetative period or reduction in vigor of the host as a result of leaf blotch.

An answer to the second question was sought in the weather records for seasons of exceptional prevalence of the stem-spot phase, and in the influence of temperature on infection by *Cladosporium paoniae*. Because of the fragmentary data available on the prevalence of stem spot in different localities and years, no well-marked correlations with weather conditions were expected. In the vicinity of Washington, D. C., the spring of 1929 was outstanding in the last 15 years for the prevalence of this disease. Peony shoot growth is most active here during April and early May. The mean temperature for April, 1929 ( $57.6^{\circ}$  F.), was the highest, and the precipitation in quantity and frequency was the greatest, except for one year, in this 15 year period. In the vicinity of Vincennes, Indiana, an important area of commercial peony culture, there was an outstanding occurrence of stem spot in 1932. The mean temperature there for April ( $57.6^{\circ}$  F.) and May ( $67.0^{\circ}$ ) was above normal, i.e., there was an "early spring," but the weather was not exceptionally wet. The outbreak of stem spot at Concordville, Pennsylvania, occurring in 1938, coincided with the warmest April in the last 15 years ( $53.7^{\circ}$ ) but precipitation was deficient. Doubtless many other factors influence the development of this disease, but temperature

appears to be one of the most important. An experienced peony grower writes, "The worst infection I have seen was of flowers from the South, and in trips to the South, I see it nearly everywhere there are peonies. No exhibit at the National Peony Show in Lansing, Mich., in 1938, was entirely free from it. I have never seen more than a slight infection anywhere north of Central Pennsylvania."

Experimental inoculations with *Cladosporium paeoniae* were successful within the temperature range 10° to 27.5° C., and the lesions were similar in appearance and size from 14° to 22°. At 10° there was a definite increase in the latent period of infection and a decline in the growth rate of lesions. The thermal range of pathogenicity of *C. paeoniae*, therefore, includes the temperatures prevailing during the period of peony-shoot growth in spring, and there is a critical point (near 14° C. or 57° F.) where the growth rate distinctly rises.

#### Control

Field experiments by a peony grower at Concordville, Pennsylvania, and pot experiments by the writer, showed that the infected shoots of the preceding year were the main source of contamination, the soil being secondary. Field plants, which were cut back to the ground in September and October, 1938, were relatively free from stem spot in 1939, and in pot experiments they were quite as healthy as plants produced from roots washed free of soil. Plants left over winter with the tops in place developed stem spot in the spring, both in the field and in pots. Placing infected stems in a previously healthy planting in early March resulted in the appearance of stem spot on surrounding plants in May, with severe infection in a radius of about 2 feet, moderate infection up to 10 feet, and occasional infection up to 20 feet. Evidently the practice by some growers of leaving the old stems in place over winter, for the purpose of snow retention, involves a serious risk of communicating stem spot to the succeeding crop.

#### Varietal Susceptibility

The marked susceptibility of the variety Avalanche has been mentioned. Mon. Bastien Le Page, a discarded commercial variety, was the only peony in a small variety plot maintained at this Station that developed stem spot in 1939. Dr. J. J. Styer of Concordville, Pennsylvania, is the authority for the statement that weak-stemmed varieties, including most reds, and all medium or dwarf growers are susceptible. The varieties Festiva Maxima and Mon. Jules Élie, which are vigorous and thick-stemmed, are but little affected.

#### SUMMARY

A red-spot disease of the stems, foliage, and flowers of peonies is widely distributed in commercial plantations, but is of infrequent occurrence in a severe form. It sometimes seriously disfigures the plants and may destroy their value for flower cutting.

Its etiological connection with *Cladosporium paeoniae* has heretofore been suspected but not definitely established. It also has been confused at times with the disease first described as anthraenose, but the cause of anthraenose has never been definitely established.

In the search for the cause of stem spot it was found that isolates of *Glocosporium fructigenum* from peony and from *Rubus* may infect peonies as wound parasites. Two other fungi, *Pezizella lythri* and a budding fungus, not further identified as yet, are also pathogenic to peonies, and may cause distinctive stem and leaf diseases. *Cladosporium paeoniae* is considered the principal etiological factor, its restricted development on young stems being due probably to its low virulence on tissues that are in active growth. The profuse character of infection, even though the lesions are small, makes this stem spot a significant disease on peonies grown for flower cutting.

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### CROWN GALL OF PEACH IN THE NURSERY

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#### INTRODUCTION

Crown gall (*Phytomonas tumefaciens* Smith and Townsend) is one of the most serious diseases on peach in the nursery. In the United States, the disease is particularly prevalent and severe in some sections of the southern and western States. No data are available for estimating the losses occa-

sioned by it, but, where the disease is prevalent, 50 per cent of the trees frequently are discarded, and brush heaps containing thousands of trees having galls like that shown in figure 1, A, are a not uncommon sight. Literature references to this disease in the United States begin about 1890, but undoubtedly the disease occurred many years before that time. Butz<sup>1</sup> in 1902, cites instances where entire blocks of nursery peach trees were destroyed because of crown gall.

Despite the large financial losses caused by this disease, no serious attempts at control have been reported. The standard recommendations have been based on the principle of rotation with crops of nonsusceptible plants, but no reports on the results of this method have been found in the literature.

Limited observations indicate that two important characteristics of this disease appear to be (1) the relatively greater severity in regions known to have alkaline soils, and (2) the localization of the majority of the galls at the region of the root-stem junction, *i.e.*, at the "crown" of the roots. These factors were taken into consideration in seeking means of control by attempting to devise methods whereby (1) noninfested soils could be maintained in that condition; (2) infested soil could be rid of infestation; and (3) the tissue of the seedling could be protected at the region most vulnerable to infection.

#### PRELIMINARY OBSERVATIONS AND EXPERIMENTS

The effect of the hydrogen-ion concentration of the soil on the amount of crown-gall infection had been investigated, and the results<sup>2</sup> indicated that liming a relatively acid (pH 5.5) soil greatly increased the amount of infection. This experiment has been repeated and will be discussed under the experiments of 1939.

In connection with the question as to why there is a rather general localization of galls at the region of the root-stem junction, as shown in figure 1, A, peach pits were germinated in the greenhouse and observations made on early stages of growth. It was found that small lesions, probably due to bruising, were present, particularly on the main axis near the cotyledons, in numbers sufficient to account for the presence of numerous galls at this region if these wounded areas became infected. Figure 1, B, illustrates a germinating seed before the cotyledons have emerged from the stony coat (endocarp). Although many pits do not have such a pronounced projection, it is apparent that comparatively slight pressure by the pointed tip of the hard seed coat would result in injury to the very tender tissues of the seedling. Figure 1, C and D, shows wounds that occurred incident to sprouting. That such wounds might serve as infection courts was indicated by the large percentage of seedlings that exhibited galls at the

<sup>1</sup> Butz, G. C. Crown gall. Pennsylvania Agr. Exp. Stat. Ann. Rpt. Part 2 1901/02: 405-414. 1902.

<sup>2</sup> Siegler, E. A. Relations between crown gall and pH of the soil. *Phytopath.* 28: 858-859. 1938.



FIG. 1. A. Typical crown gall on 1-year budded peach tree. B-D. Stages in germination of peach. In B, note possibilities for injury to the tender tissues at the region near the cotyledon petioles by the stony endocarp. In C and D, note lesions incident to normal germination. E. Gall no young seedling resulting from natural infection.

cotyledonary region, as shown in figure 1, E, when pits were planted in soil infested with the crown-gall organism.

Preliminary experiments in control, therefore, were made in an attempt to protect the tender tissues of the germinating seed from infection. The seeds in early stages of germination were treated with various antiseptics and disinfectants, including calomel, mercuric chloride, thymol, formaldehyde, "formofume," sulphur, sodium hypochlorate and cuprous oxide. The results of these preliminary experiments were not conclusive because of great variability among the several treated and control plots. However, soil treatment with sulphur and seed treatment with calomel were deemed worthy of further trial.

The more extensive experiments conducted in 1939 were planned mainly to observe the effect of varying the pH of the soil and the effect of applying a disinfectant to the pits, on the amount of infection on 1-year seedlings.

#### Materials and Methods

Hydrated lime was used when it was desired to make an acid soil alkaline. Sulphur (commercial dusting) and ammonium sulphate were used in an attempt to acidify the soil in certain test plots that had been previously made alkaline by liming.

Calomel (U. S. P.) was used on the pits, which were comparatively free from dirt, at the rate of 4 oz. to 1 gal. of water. The pits were held in an open-mesh cloth bag and dipped in the well-stirred suspension for several minutes to permit a thorough coating. They were then permitted to surface-dry before planting.

The soil in every plot, including the "no treatment" or "check" ones, was artificially inoculated by pouring heavy water suspensions of the crown-gall organism into a 6-inch-wide shallow furrow in which the pits were to be planted. The manipulations were such as to insure a wetting of the soil with the inoculum to a depth of approximately 2 in. below the surface of the soil in the furrow in which the pits were to be planted.

#### Procedure

In the plots to be tested for the pH effect (Experiment 1), the limed plots received an application of hydrated lime in a shallow 6-inch-wide furrow, and 8 days later all plots were inoculated with the organism.

All the plots to be tested for the effect of calomel, sulphur, and ammonium sulphate and their "check" plots (Experiment 2) were first given an application of lime, and, 8 days later, were inoculated with the organism. In the plots treated with sulphur and ammonium sulphate the chemicals were sprinkled directly onto the pits in the furrow.

The pits were planted in November, 1938.

#### RESULTS

The details of experiment No. 1, designed to test the effect of the pH of the soil on the amount of crown-gall infection, are shown in table 1.

TABLE 1.—*Comparison of the amount of crown gall on 1-year peach seedlings grown in (1) acid and in (2) alkaline soil*

Row	Treatment	Number of trees	Number of galled trees	Percentage galled trees	<sup>a</sup> pH of soil
1	None	1291	100	8	5.8
2	Limed	1168	713	61	8.5
3	None	835	91	11	5.9
4	Limed	1321	754	57	8.5
5	None	1135	109	10	5.8
Total	None (3 rows)	3261	300	9	5.8
"	Limed (2 rows)	2489	1467	59	8.5

<sup>a</sup>All pH determinations were made by W. F. Kosar on a quinhydrone electrode.

The results of this experiment confirm those of the preceding year and furnish additional evidence that liming an acid soil apparently makes for conditions favorable for growth of the organism and results in an increased amount of crown gall.

The seedlings in the limed rows made, on an average, slightly better growth (approximately 2 inches) and had slightly darker foliage than those on the non-limed rows. When this condition became apparent, a light side dressing of nitrate of soda was applied to 250 of the seedlings in Row 1. As anticipated, there was a prompt growth response and, by digging time, these trees in this plot were as large as those in any of the limed rows. The total stand was approximately 50 per cent.

In experiment No. 2, an attempt was made to control crown gall by (1) the use of calomel on the pits and by (2) applications of sulphur and ammonium sulphate to the soil. The field plan consisted of two adjacent rows, each row containing 16 plots, 12.5 ft. long. Each plot contained 150 pits and, it will be recalled, the soil in all the plots in these two rows had been limed and then inoculated with the crown-gall organism prior to planting the pits.

The data on the experiment (Table 2) are arranged so that the relative positions of the plots can be readily visualized; the lots that were opposite each other, but in adjacent rows, are on the same parallel lines.

As shown in this table, the stand of trees was very uniform, with the exception of lot 8. In this experiment excellent control was secured by the use of calomel alone (6 per cent galled trees in the 4 plots), as compared with 71 per cent galled trees from the adjacent nontreated check plots 1, 11, 18, 28. Typical trees, classed as clean and galled, are shown in figure 2, A and B, respectively. The fact that the trees in row 2 made on an average approximately 2 to 4 inches more growth and had darker foliage than the trees in row 1 is not shown in the table. The better growth was attributed to leachings from manure that had been spread on higher ground about 8 feet away from row 2. As might be anticipated, the height and caliper of the trees were greatest in all the plots treated with ammonium sulphate. The total stand was 56 per cent.

In contrast to the effect of the calomel treatment alone, this treatment





FIG. 2. One year peach seedlings typical of those in experiments, showing the characteristic "bend" at the root-stem junction. Note tap roots in comparison with branched roots (Fig. 1, A). A. Typical of seedlings classed as "clean." B. Typical of the seedlings classed as "galled." Of the galled trees, 90 per cent had galls located at the "bend" at the root-stem junction.

in combination with either sulphur or ammonium sulphate and the plots receiving the latter materials singly, failed to yield consistent results. So many factors may be involved in these particular treatments that a discussion of them is not warranted at present.

As indicated by the pH readings of the so-called nontreated plots, it would appear that the application of lime resulted in a degree of alkalinity seldom encountered in nurseries. The pH of the land in the immediate vicinity of these two rows was approximately 5.7. Attention is called to the fact that all the pH readings were made from composite samples taken at the time the seedlings were dug, and that the readings above pH 8.0 are not considered reliable. The fact that sulphur particles were abundant

in the sulphur-treated plots at the time of sampling would tend to explain discrepancies in pH readings, as exemplified in plots 23 and 29. Applications of ammonium sulphate had no residual acidifying effect 10 months later. It is possible that applications with this material when the pits are cracking in the spring might result in effective acidification for a short period.

#### DISCUSSION

The results of these experiments are encouraging for the practical control of peach crown gall in the nursery. Obviously, repeated tests under varying conditions should be made before the results are considered conclusive. The experiments that showed an increase in crown gall as a result of liming an acid soil have confirmed the results obtained in the preceding season and, therefore, are considered more conclusive. In regions where the soil is heavily infested with the crown-gall organism a treatment of the pits with calomel can be made with negligible expense and is at least worthy of a trial.

No data are available on the question as to how closely soil infestation is limited to soils near to or on the alkaline side, or, more especially, as to occurrence of heavy infestation in relatively acid soil. It is hardly necessary to point out that failure to secure satisfactory infestation artificially in an acid soil does not warrant the conclusion that this same soil might not become infested by natural means. However, the known proclivities of other pathogenic soil organisms make it permissible to assume that the presence and the virulence of the crown-gall organism may be influenced by the pH of the soil. Recently, Hornbostel<sup>3</sup> has reported that certain organic mercury compounds have greater bactericidal effect on the crown-gall organism when used in media of comparatively low pH values, and Sherbakoff<sup>4</sup> has reported experiments on the use of sulphur as an acidifying agent to control "true crown gall" on apple grafts.

In these experiments the attempts to change radically the pH of soil which had been made alkaline by liming, yielded inconsistent results. Further experimentation is necessary to learn if the amount of infestation occurring in alkaline soils actually will be appreciably reduced if, by certain treatments, these soils can be made relatively acid. The important question as to the proper alterant for any given soil lies beyond the scope of this report.

In situations where the organism may persist in the soil despite all efforts to eliminate it, the problem of control is mainly concerned with protecting the peach seedling where and when it is most vulnerable to infection. Field observations and preliminary experiments indicate (1) that a large proportion of trees are attacked about 2 inches below the ground

<sup>3</sup> Hornbostel, W. Die Beziehungen zwischen Bodenreaktion und Wirkung queck-silberhaltiger Bodenentseuchungsmittel auf den Wurzelkropferreger *Pseudomonas tumefaciens* Smith et Townsend. *Ztschr. f. Pflanzenkrank.* 49: 77-93. 1939.

<sup>4</sup> Sherbakoff, C. D. Effect of soil treatment with sulphur upon crown gall in nursery apple trees. *Phytopath.* 15: 105-109. 1925.

level, which is at the general region of the root-stem junction, and (2) that severe mechanical injury to the emerging root frequently occurs in early stages of seed germination, causing not only many wounds but frequently the destruction of the growing point, as a result of which many trees exhibit branched roots instead of a normal tap root. The large size and general characteristics of the galls, usually encountered in nursery trees, also indicate that they are 2 years old. Therefore, although other supporting data are not at hand as proof, it can be assumed that wounds at the top or proximal part of the young root system serve as important infection courts for the organism, and that control measures, predicated on this assumption, should be concerned with attempts at (1) elimination of such wounds and (2) protection of the tissue of the young seedling by the antiseptic or disinfectant action of a suitable material. In some sections of the country the practice of planting the sprouted seed in early spring undoubtedly results in a considerable amount of wounding in comparison with the amount that results when the seed is permitted to germinate "in place." In these sections it may be found practicable to hold the seed in a dormant but after-ripened condition in cold storage, so that the pits will not be "cracked" when planted.

The results obtained in these experiments with the use of calomel as a protectant are highly encouraging, but it should be emphasized that these experimental plantings differ in many features from conditions in the commercial peach nursery. These experiments were designed to establish principles for control and, as such, have their value, but such clean-cut results under commercial conditions would not necessarily be obtained. For example, it will be recalled that in these experiments the inoculum was applied only in the immediate vicinity of the pits. There was apparently very slight diffusion of the organism in the soil because all of the galls were confined to the main axis of the root in a region about 2 inches in length, beginning at the root-stem junction; no galls were found on the smaller lateral roots. Moreover, despite the fact that the seeds were planted in the fall and germinated "in place," some wounding undoubtedly occurred; but injury, sufficient to kill the growing point of the young root was evidently infrequent, because practically all of the seedlings had tap roots. By count, however, 90 per cent of the galls on the affected seedlings were located at the "crook," which is formed at the region of the root-stem junction, due to curvatures occurring in early stages of germination. This supports the field observations concerning the location of the majority of the galls and demonstrates the susceptibility of the tissues at this region, presumably while they are still soft and succulent, if not actually wounded.

The fact that the seedlings in these experiments were dug at the end of the first growing season should also be taken into consideration in evaluating these results and in forecasting their applicability to commercial practice where the roots remain in the ground one year longer. Regardless, however, of the number of new infections that may occur during the

second season, it is apparent that protection up to that time is a prerequisite for control.

#### CONCLUSIONS AND SUMMARY

Crown gall (*Phytoponas tumefaciens* Smith and Towns.) is, in the nursery, one of the most serious of peach tree diseases. The majority of the galls are located at the region of the root-stem junction at the crown of the root system.

The disease is very prevalent in regions where it is the practice to plant the seed after it has sprouted. This practice undoubtedly results in injury more severe than when the pits are planted in the fall and thus are permitted to germinate in place. Even in the latter procedure, however, numerous small lesions occur on the tender tissues during the very early stages of growth. Presumably, these lesions serve as infection courts; but, in any event, the tissues of the roots, particularly in the general region of the root-stem junction, are very susceptible to infection.

Another factor in the etiology of this disease is the general prevalence of the organism in regions in which the soils are relatively alkaline.

The experiments reported here were concerned mainly with securing confirmatory data on the effect of the pH of the soil on the amount of infection and with attempts to lower the pH of soils, made alkaline with lime, by applications of sulphur and ammonium sulphate. In addition, attempts at control were made by dipping peach pits in a heavy suspension of calomel before planting in an endeavor to protect the tissues of the seedling from infection during the early stages of germination.

The results of the experiments furnish additional evidence that a much larger amount of infection occurs when alkaline soils are artificially inoculated than when acid soils are artificially inoculated. The amount of infection was 59 per cent in the limed plots and 9 per cent in the nonlimed plots.

As a result of one season's trial the plots containing the calomel treated pits showed 6 per cent infection in comparison with 71 per cent on 4 control plots.

Regardless of the many factors that should be given consideration in evaluating these results and of the precautionary statements that naturally should qualify the results of preliminary experiments, it is believed that this attempt to establish the important factors incident to infection should eventually lead to adequate control measures.

In view of these results, it would seem advisable to avoid the excessive use of lime on soils in peach nurseries where crown gall is a factor. Obviously, an acid condition as is compatible with satisfactory growth is desired. In addition, treatment of the hard, uncracked pits, with a strong water suspension of calomel (4 oz. to 1 gal.) at planting time is worthy of a trial to test the efficacy of this treatment under various conditions.

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## THE INHERITANCE OF IMMUNITY FROM MILDEW (*BREMIA LACTUCAE*) IN LETTUCE

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Milbrath (4) was the first investigator to point out the serious damage to the commercial lettuce crop caused by downy mildew (*Bremia lactucae* Reg.). He stated the "climatic conditions in California are favorable and conducive to the growth of *Bremia lactucae* in the field." This statement is particularly true of the Salinas-Watsonville area, which is the State's major lettuce-producing region. Another troublesome characteristic of the disease is the fact that it continues to develop in shipment. As a result, lettuce attacked by mildew arrives on the markets in poor condition.

Milbrath tested a number of varieties, and found considerable difference in susceptibility to mildew among them. The New York variety proved the most susceptible; Hanson and Iceberg were more or less resistant; none of the varieties tested was immune from the disease.

In 1923, the senior writer (2) reported that a large number of varieties had been tested, and 9 were found to be immune from mildew in both California and Florida. These 9 varieties were of European origin, and, in general, unsuited to cultivation under California conditions. They were crossed with the very susceptible, but commercially popular, variety New York. All first generation hybrids were immune from mildew. Segregation in F<sub>2</sub> closely approximated 3 immune plants to 1 susceptible, suggesting that immunity from the particular physiologic race of *Bremia lactucae* involved was governed by a single dominant gene.

In a series of inoculation experiments on the 9 varieties mentioned above, Jagger and Chandler (3) were able to show very definitely the existence of at least 4 distinct physiologic races of the fungus; race 1, found at Sanford, Florida, and Chula Vista, California; race 2, found in England; race 3, in the Imperial Valley, and race 4, in the Salinas Valley, California. One variety, *Romaine blonde lente a monter*, obtained from France seemed immune from all forms of the fungus encountered. This variety, according to Vilmorin-Andrieux & Cie (9), originated in Southeastern France. It is a typical Cos type lettuce, with pale green, elongated, spatulate leaves. This variety was crossed with several strains of New York or "Iceberg" type lettuce. Through selection and further backcrossing to the New York type, a number of commercially desirable strains resistant to the then known physiologic races of *Bremia lactucae*, and superior to the original New York variety, were released to the industry (Imperial F, Imperial D, Imperial 152, Imperial 615, Imperial 847, etc.).

In 1932, another physiologic race of *Bremia lactucae* appeared in the Salinas Valley, for all of the strains listed above as immune were attacked

<sup>1</sup> Deceased February 16, 1939.

by the parasite. In an extensive series of tests 2 varieties and a selection of *Lactuca scariola* from Russia proved immune from physiologic race 5 of *B. lactucae*. Again, the immune varieties were of European origin; two were primitive, as far as heading qualities were concerned, and commercially useless.

In this paper, data are presented indicating 1, that immunity from physiologic race 5 is dependent upon a single dominant gene; 2, that it is possible to combine genes for immunity with good commercial qualities in lettuce, by crossing, backcrossing, and selection; 3, that genes for immunity are found in the more primitive, non-heading or loose-heading types of lettuce.

#### MATERIALS AND METHODS

Two types of lettuce immune from mildew were used in these experiments. Their source and description follow.

1. *Grosse blonde d'hiver Bourguignonne*. Obtained from Vilmorin-Andrieux & Cie. A butter-head type. Leaves, smooth with regular margins; plant and head, entirely light green; heart, buttery yellow. Similar to the variety Unrivalled, but probably a little darker green and possibly a little larger. Under our conditions it "tip burns" badly, but is immune from mildew.

2. *Lactuca scariola*.<sup>2</sup> Obtained through the division of Plant Exploration and Introduction from Russia (P.I. No. 104854). Strong, vigorous plants with pinnately lobed leaves; older leaves, bristly hispid along the midvein; glaucous otherwise; the younger leaves are entirely smooth; no red pigment in either stems or leaves. The young plants form a small rosette of leaves and immediately produce 4 or 5 side shoots, the latter becoming seed stalks of uniform height (24 to 30 in.).

The French variety and *Lactuca scariola* were used directly in the crosses reported in this paper. In the last 3 families listed in table 1, a homozygous resistant segregate derived from an original cross between a plant of *L. scariola* and a susceptible type, with several intervening generations of selection and backcrossing, was developed as a parent for further crosses. It is important to note that resistance in this case originated with the plants of *L. scariola* from Russia.

The susceptible varieties used in these experiments were Imperial F, Imperial D, Imperial 615 and Imperial 847. They are commercial sorts of the "Iceberg" type, and were developed and released by the U. S. Department of Agriculture. At the time of their release, physiologic race 5 had not appeared, and they were immune from the forms of *Bremia lactucae* then present. They are firm, well-folded, and of good quality when grown under conditions to which they are adapted.

Seed for plants to be scored for resistance and susceptibility was planted in the seed bed and the young plants were inoculated with a water suspension of spores of the fungus as soon as the cotyledon leaves became fully expanded. Inoculum was prepared by washing spores from mildew-infected plants. Small cultures of such plants are maintained continuously in order to have a readily available supply of the pathogen for inoculation purposes. After the results of the first inoculation became apparent, the susceptible plants were removed and the remaining plants again inoculated. This second inoculation made it fairly certain that very few potentially mildew-susceptible plants escaped inoculation.

<sup>2</sup> The evidence is quite conclusive that the cultivated varieties of lettuce have been derived from *Lactuca scariola* (1).

## RESULTS

From crosses made between susceptible commercial varieties and homozygous immune ones, immune  $F_1$  plants were obtained. The data obtained for segregation of mildew immunity in the  $F_2$  progenies from these crosses are presented in table 1.

TABLE 1.—*Segregation for mildew immunity in  $F_2$* <sup>a</sup>

Cross	Family No.	Diseased	Healthy	Total
F × French variety	3158	147	436	583
D × <i>Lactuca scariola</i>	4502	143	470	613
847 × resistant plant	40018	93	257	350
847 × " "	13279	71	224	295
615 × " "	13247	42	128	170
Totals		496	1515	2011
Calculated (3:1)		502.75	1508.25	

<sup>a</sup> Deviation = 6.75;  $\chi^2$  = 0.12646; range = 0.00 - 3.841.

The data obtained from the 5 families listed in table 1 have been subjected to the  $\chi^2$  test for homogeneity of the individual families. The results indicate that the departures from the theoretical are within the limits of random sampling. The  $\chi^2$  test for goodness to fit (3:1) has been applied to each family, independently, and to the totals. Insofar as data on the parents, the  $F_1$  and the  $F_2$ , are concerned it appears that the hypothesis of a single dominant gene accounts satisfactorily for the observations.

The  $F_3$  data are not extensive, but seem sufficient to support the single-gene hypothesis. Five  $F_2$  plants, immune from mildew, from the cross D × *Lactuca scariola*, were tested in  $F_3$ . All proved to be heterozygous (Table 2).

TABLE 2.—*Segregation for mildew immunity in  $F_3$* 

Cross	$F_2$ Phenotype	Segregation in $F_3$			$\chi^2$
		Diseased	Healthy	Total	
F × French variety	Diseased	50		50	-
F × " "	"	8		8	
D × <i>Lactuca scariola</i>	"	55		55	
D × " "	"	15		15	
D × " "	Immune	1	4	5	0.06666
D × " "	"	16	42	58	0.20689
D × " "	"	8	28	36	0.14815
D × " "	"	12	37	49	0.00680
D × " "	"	9	31	40	0.13333

In many cases young seedlings, infected with *Bremia lactucae* are weakened, and die in the seedling stage. With some care to avoid infection by secondary organisms it is possible to raise mildew-infected plants to maturity. Mildew-susceptible plants were selected out from several  $F_2$  families; the progeny from these plants were homozygous for mildew susceptibility (Table 2).

In table 3 are summarized the test-cross data. Test-cross matings were not made to the susceptible parent because it is often difficult or even impossible to determine whether a particular individual is the result of a cross or of self pollination, except by the use of markers. In testing 4 of the  $F_1$  progenies we have made use of a homozygous susceptible plant with a border of red pigment around the leaf edge as a pollen parent, mated to the heterozygous  $F_1$  plants. All cross-pollinated individuals should show red pigment of somewhat the same nature as the pollen parent. In the latter case a typical plant of the variable species *Lactuca scariola* was used. By means of this technique it is a comparatively simple matter to eliminate the self-pollinated plants. The data of table 3 have been subjected to the same tests described for table 1. The results do not deviate significantly from the expected 1:1 ratio.

TABLE 3.—*Segregation for mildew immunity in test crosses*<sup>a</sup>

Test cross	Family No.	Diseased	Healthy	Total
$F_1$ (Imp. D $\times$ <i>L. scariola</i> ) $\times$ red edge suscept.	33570	19	35	54
$F_1$ (Imp. 615 $\times$ resistant) $\times$ “	33578	21	27	48
“ “	33580	30	26	56
$F_1$ (Imp. 847 $\times$ resistant) $\times$ “	33581	28	30	58
“ “ $\times$ speckled red suscept.	33584	3	4	7
$F_1$ (Imp. 615 $\times$ resistant) $\times$ “	33586	5	5	10
“ “	33588	10	13	23
$F_1$ (Imp. D $\times$ <i>L. scariola</i> ) $\times$ <i>L. scariola</i> (suscept.)	33611	3	2	5
Totals		119	142	261
Calculated (1: 1)	..	130.5	130.5	

<sup>a</sup> Deviation = 11.5;  $\chi^2$  = 2.0268; range = 0.00 - 3.841.

#### DISCUSSION

There are two facts established by this investigation that merit further discussion, since they are of general genetic significance. They are: 1. The gene for immunity is dominant over its allele for susceptibility. 2. The plants with the dominant genetic complexes are of European origin.

The 9-chromosome species of *Lactuca* (Babcock, *et al.* (1)) are, with one exception, of European origin. Cultivated lettuce and the closely related species, *L. scariola*, belong to this group. The two facts mentioned above would seem to support Vavilov's contention (8) that the greatest diversity in form of a cultivated species is found in the vicinity of its origin, and that, during the spread of a species toward the boundary of a region, the recessive forms are singled out for survival. Conversely, the proportion of dominant genes is greater in the immediate vicinity of the center of distribution.

Of equal significance may be the fact that the immune types are more or less primitive, or nonspecialized. The highly developed, specialized heading types, *i.e.*, New York, etc., are, without exception, carriers of the recessive genes for susceptibility.

The dominant gene for immunity from mildew in lettuce parallels very closely in origin and behavior the *Fu* gene for resistance to *Fusarium* wilt described by Wade, *et al.* (10) in peas. The similarities are obvious; the genes for resistance or immunity are dominant, and are found in relatively unspecialized types; forms with dominant genetic complexes are found near the center of distribution of the species in question. It, however, is not true that all of the primitive types of lettuce either cultivated or wild (*L. scariola*), carry the dominant genes for immunity.

The recent work of Schultz and Röder (5) supports our observations that the more primitive types of lettuce carry the dominant genes for immunity. In their very extensive trials at the Experimental and Research Institute for Horticulture in Germany, they found 3 varieties of the general type of May King (May King, May King Forcing, Bottner's Forcing), which appeared to be very resistant to if not immune from the disease. These varieties are of the early forcing type, making loose, spongy heads and having buttery-texture leaves. The senior writer has found May King immune from most of the physiologic races encountered in this study, but it was susceptible to certain races in England, and in Imperial Valley, California.

Schweizer (6) has shown that there is considerable physiological specialization within *Bremia lactucae*; that is, spores from one host infect only the same host or other species of the same genus as the host. In no case was he able to cross-infect to species of another genus. By means of statistical methods, Schweizer was able to distinguish what he termed "small morphological species" within *B. lactucae*. Schultz and Röder (5) have isolated 2 physiological races of *B. lactucae* in Germany. These races were separated on the basis of differential pathogenicity to certain varieties of lettuce.

Stakman (7) lists 4 principal methods that have been suggested to explain the origin of races of phytopathogenic fungi. They are as follows: adaptation, hybridization, heterocaryosis, and mutation.

We have no critical test that would positively discredit the theory of the origin of physiological races in *Bremia lactucae* through adaptation. Indirect evidence, however, seems to indicate that it is highly unlikely. If the parasite had adapted itself to previously immune varieties, these presumably would have "lost their resistance" rather gradually over a series of growing seasons. Actually, the new physiologic races appeared suddenly, and varieties that had been immune were completely susceptible to the new forms.

It is doubtful whether either heterocaryosis or hybridization should be considered as probable methods of origin of physiological races in *Bremia lactucae*, for the reason that the sexual stages of the fungus have never been observed in this locality. Our knowledge of this subject is far from complete; it is entirely possible that a careful examination might disclose sexual reproduction in the life history of the parasite.

That physiological races of *Bremia lactucae* have originated through mutation seems reasonable from circumstantial evidence. The manner in which they first appeared, and the fact that resistance to at least two of them is controlled by single gene differences would indicate that they may have originated through mutation.

These observations make the assignment of producing disease-resistant plants a difficult one. The best opportunity for success seems to be in maintaining a large collection of types of the host species, including primitive types from near the center of origin of the species on the chance that some of them will carry genes for resistance to new physiologic races, as these races are discovered, and become economically important.

From general observations there is no evidence of linkage between genes for immunity, and those for the various pigments, morphological structures, or physiological characteristics of the strains or varieties of lettuce used in this study. For this reason it is a comparatively simple matter to combine the genes for immunity with those for desirable commercial qualities.

#### SUMMARY

The occurrence of 5 physiologic races of *Bremia lactucae* is recorded. There is evidence of the existence of as many as 6 or 7 races of this fungus that attack cultivated lettuce.

The inheritance of immunity from physiologic race 5 is described in detail. Immunity is dependent upon a single dominant gene.

Dominant genes for immunity have been found only in the more primitive types of lettuce. These types occur in Europe, and presumably come from near the point of origin of cultivated lettuce.

There is no evidence of linkage between genes for immunity and any of those for the various morphological characters found in cultivated lettuce.

It is suggested from indirect evidence that physiologic races in *Bremia lactucae* originate through mutation.

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## HYDROXYL-ION CONCENTRATION OF THE SALIVA OF PARTLY DESICCATED BEET LEAF HOPPERS

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The beet leaf hopper, *Eutettix tenellus* (Baker), is the only known vector of the curly-top virus. The virus exists in the body of the insect for long periods (3, 6). Knowledge of the chemical environment of the virus within the leaf hopper is, therefore, of interest. Earlier investigations have shown that the salivary secretions of normal beet leaf hoppers are distinctly alkaline, and that the blood or body fluid is slightly alkaline (4, 1). Bennett and Wallace (3) came to the conclusion that the blood of the insect is the main reservoir of the virus. The phloem of sugar beets, which is a favorable environment for the virus, is slightly alkaline (5).

The object of this paper is to present data on the hydroxyl-ion concentration of the salivary secretions of starved and, consequently somewhat desiccated leaf hoppers, and to correlate the findings with some facts previously observed.

### METHODS

In order to determine the equivalents of hydroxyl ions, secreted during feeding by the salivary glands of partly dessicated leaf hoppers, it was necessary to allow the insects to feed on small drops of a slightly buffered liquid for a definite period.

A buffer solution, consisting of 0.01 normal HCl and 0.09 normal KCl, was diluted 10 times with distilled water to make a feeding solution. The object of diluting the buffer solution was to reduce its buffer capacity and at the same time to increase its pH to a point beyond its most effective buffer range. Sufficient sucrose was then added to bring the concentration to 2 per cent. This feeding solution, freshly prepared, had a pH of 3.07. Calculations from the buffer curve show a maximum buffer value of  $1 \times 10^{-5}$  between pH 3.31 and 3.11 and a minimum buffer value of  $1.28 \times 10^{-4}$  between 4.48 and 5.12. The feeding solution had a buffer value of  $3.7 \times 10^{-4}$  over the entire pH range through which it was used. The feeding solution had sufficient buffer capacity to maintain a constant hydrogen-ion activity, yet the buffer capacity was so low that the addition of an extremely small

quantity of hydroxyl ions would shift the pH considerably. A fresh feeding solution was prepared when the pH was found greater than 3.65 or when evidence of bacterial growth appeared.

Female leaf hoppers, which had been kept without food or water for 18 to 24 hours at room temperature, were placed singly in individual feeding chambers previously described (4). When a leaf hopper in search of food punctured the paraffine membrane, a drop (0.01 cc.) of the feeding solution was placed on the membrane directly over the insect. In this way it was possible almost at will to induce desiccated leaf hoppers to feed.

A leaf hopper was allowed to feed for a definite period of time, then the drop was transferred to another paraffine membrane for the pH determination. The transfer was accomplished by inverting the feeding chamber. In this position the drop would still adhere to the underside of the membrane. Another paraffine membrane, stretched across the mouth of a small vial, was raised until it came in contact with the drop and then lowered. The drop would then adhere to the lower membrane. The feeding chamber was next righted and another drop placed over the leaf hopper whose mouth parts were still protruding through the membrane. In this way a leaf hopper could feed continuously, being interrupted only for about 10 seconds, while the drop of feeding solution was being replaced by a fresh one.

The drops were changed at regular intervals until the leaf hopper refused to feed. By changing the drop at regular intervals it was possible to follow the rate at which the insect injected hydroxyl ions into the feeding solution.

The pH determinations were made alternately (with the quinhydrone electrode) on the drops on which the leaf hoppers had fed and on control drops of the same volume that had stood in the open and under the same conditions for the same length of time. The electrode consisted of a platinum wire ground down until the tip was approximately  $60\ \mu$  in diameter. A fine capillary tube filled with agar saturated with salt served as the salt bridge.

The pH of the control drops was constant. As many as 20 tests on the control drops would be made during one day. The probable error of the mean for any series of tests made on the same day was never greater than  $\pm 0.04$  pH unit.

#### RESULTS

The pH of the saliva of 75 leaf hoppers was tested in the manner described above. The saliva of most of the insects was tested more than once. Nearly all of the leaf hoppers were tested as long as they would feed, the feeding drops being changed at regular intervals.

As many as 20 tests were made during the feeding period of a single leaf hopper. In these tests the feeding drops were changed every 2 minutes; in other tests the feeding drops were changed every 5 minutes. The points in figure 1 show the pH of the feeding drops after the injection

of hydroxyl ions by 4 leaf hoppers, A, B, C and D, and are typical of the results obtained. Each point represents a drop on which leaf hopper A or B, respectively, fed for a period of 2 minutes, while each point, with one exception (leaf hopper C, drop 6), represents a drop on which leaf hoppers C or D, respectively, fed for 5 minutes.

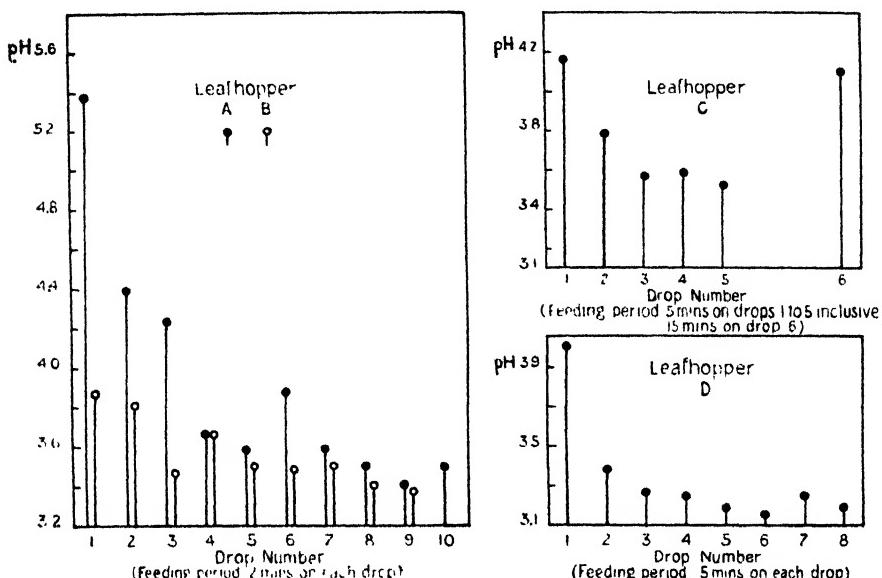


FIG. 1. The change in pH of 4 series of drops upon which 4 leaf hoppers were separately fed. The ordinates indicate the pH of the individual drops at the conclusion of the feeding period. The pH of the drops before feeding started was 3.2 for leaf hoppers A and B and 3.1 for leaf hoppers C and D.

The leaf hoppers were able to increase the pH of the feeding solution in all cases where they were known to have fed, regardless of whether or not they had been previously desiccated. It is evident from the figure, however, that the equivalents of hydroxyl ions injected was generally less in each successive drop. During the latter part of the feeding period when the leaf hoppers were no longer in a desiccated condition hydroxyl ions were still being excreted.

The equivalents of hydroxyl ions injected into each drop of feeding solution was determined from the buffer curve.<sup>1</sup> The pH that would be obtained, if the same number of equivalents of hydroxyl ions were injected into the same volume of distilled water, was calculated. These data are shown in the last 4 columns of table 1.

<sup>1</sup> The pH was determined (with the quinhydrone electrode) on 5 ml. of the feeding solution after each addition of small increments of 0.001 normal sodium hydroxide. The maximum volume of sodium hydroxide added between pH determinations was 0.50 cc. As the buffer capacity decreased, smaller increments of sodium hydroxide were added until, at the point where the buffer value was least, only 0.01 cc. of sodium hydroxide was added between pH determinations. The pH values were plotted against the total volume of sodium hydroxide added. The volume of 0.001 normal sodium hydroxide required to bring 5 cc. of the feeding solution to the same pH value as that of the drop on which the leaf

It is evident from the figure and the data presented in table 1 that the equivalents of hydroxyl ions injected into the first, second, and third drops are large compared to that injected into the remaining drops.

TABLE 1.—*The secretion of hydroxyl ions by partly desiccated beet leaf hoppers while feeding on a slightly buffered sugar solution*

Drop (0.01 cc.)	OH ions secreted into each drop			Calculated pH of drop of water containing same equivalents of OH <sup>-</sup> ions					
	A <sup>a</sup>	Leaf hopper		D <sup>b</sup>	A	Leaf hopper		D	
		B <sup>a</sup>	C <sup>b</sup>			B	C		
<i>No.</i>				<i>10<sup>-10</sup> Equivalents</i>				<i>pH</i>	
1	70	44	58	54	10.85	10.64	10.76	10.47	
2	58	42	47	23	10.76	10.62	10.66	10.36	
3	54	22	36	14	10.73	10.39	10.56	10.14	
4	34	33	38	12	10.53	10.51	10.58	10.01	
5	30	24	33	6	10.48	10.38	10.52	9.78	
6	44	24		3	10.65	10.38	10.77	9.50	
7	30	24		12	10.48	10.38		10.08	
8	24	18	57	6	10.38	10.26		9.78	
9	18	15			10.26	10.18			
10	24				10.38				
Total	368	246	269	130	11.59 <sup>c</sup>	11.39 <sup>c</sup>	11.43 <sup>c</sup>	11.11 <sup>c</sup>	

<sup>a</sup> Leaf hoppers fed 2 minutes on each drop.

<sup>b</sup> Leaf hoppers fed 5 minutes on each drop, with one exception. Leaf hopper C fed 15 minutes on drop number 6.

<sup>c</sup> The (calculated) pH that would result if all the hydroxyl ions, secreted by the leaf hopper, were concentrated in one drop (0.01 cc.) of water.

If the leaf hoppers are allowed to feed for longer periods on the same drop of feeding solution, the equivalents of hydroxyl ions injected are generally greater. A typical example of this is shown by leaf hopper C. This insect, after feeding 5 minutes on each of 5 drops, was allowed to feed 15 minutes on the 6th drop.

#### DISCUSSION

If it is assumed that the saliva of the leaf hopper is diluted 1000 times when injected into a drop (0.01 cc.), then  $1 \times 10^{-5}$  cc. of leaf-hopper saliva<sup>2</sup> containing the  $70 \times 10^{-10}$  equivalents of base was injected by leaf hopper A into the first drop of feeding solution. If these assumptions are used, calculations show that the total concentration of bases in the leaf hopper's saliva would be about 0.7 normal.

The actual volume of saliva injected into the drop of feeding solution is not known. Nevertheless, the volume of saliva that was injected into each drop contained the number of equivalents of hydroxyl ions shown in table 1.

hopper had fed, was read from the buffer curve. The equivalents of hydroxyl ions required to change 0.01 cc. (one drop) of the feeding solution over the same pH range was then calculated. In this way the equivalents of hydroxyl ions injected into each drop of feeding solution by the leaf hoppers were determined.

<sup>2</sup> The average weight of a female leaf hopper is approximately 0.0015 gram. If the leaf hopper ejects  $1 \times 10^{-5}$  cc. of saliva (density assumed to be 1.0) into each drop of feeding solution this volume would amount to approximately 0.66 per cent of its weight.

As proteins in general are quite inefficient buffers and their presence in the saliva probably would have little effect on the buffer capacity, it appears that the leaf hopper's saliva is highly buffered with inorganic salts.

It is evident that the pH of the nondiluted saliva would be greater than when highly diluted. From this it appears that the pH of the nondiluted saliva of desiccated leaf hoppers would equal at least the values shown in table 1, or higher. The dissociation of sodium and potassium carbonates and the tertiary phosphates of sodium and potassium are sufficient to account for these pH values.

It seems logical that the total salinity and the pH of the blood of the leaf hopper must be maintained within narrow limits. If this be true, then, as desiccation proceeds, it seems reasonable that water would not be spared to eliminate the salts through the alimentary tract. If the salivary glands are the reservoir for the excess salts of the body fluid, then it would seem logical that the salts would be eliminated most rapidly by ejecting saliva containing the salts during feeding.

Bennett and Wallace (3) found that, when leaf hoppers were kept without food and water 18 hours or longer and then allowed to feed 6 minutes on each of 20 seedling beets consecutively, the percentage of infection was low for the first period, increased somewhat in the second, and rose in the third to a level that was then maintained. They state: "Starvation combined with a certain amount of desiccation may have brought about certain changes that tended to inactivate any virus that might have been held in the salivary glands; hence the usual amount of infection could have been produced only after these conditions were corrected." It may be that the high concentration of hydroxyl ions in the saliva of partly desiccated leaf hoppers results in inactivation of much of the virus then in the salivary glands.

#### SUMMARY

The saliva of beet leaf hoppers that had been kept without food or water 18 to 24 hours contained a high concentration of hydroxyl ions. Measurements and calculations show that the pH of normal leaf hoppers' saliva is greater than 10 and may reach approximately 11 in the saliva of desiccated leaf hoppers.

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# SWEETCLOVER, A PROBABLE HOST OF TOBACCO STREAK VIRUS<sup>1</sup>

W. D. VALLEAU

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Streak, a virus disease of tobacco, has been observed in Kentucky since 1923 but until the past three years outbreaks were so rare that it was given but little attention. In 1932 the disease was named and photographs from an outbreak that occurred in 1923 in Marion County, Kentucky, in topped tobacco were published.<sup>2</sup> In 1933 E. M. Johnson transmitted the virus from an affected plant in the field to a plant growing in the greenhouse by grafting.<sup>3</sup> The graft was made July 16 and symptoms developed in a sucker August 15, 1933, thus proving the virus nature of the disease. In 1935 J. Johnson<sup>4</sup> published a comprehensive study of the disease and also named it streak. During the past 3 years reports of serious outbreaks of the disease have been received from county agents and farmers. Three fields have been seen in which streak was scattered throughout extensive plantings with percentages of 10 to more than 50 in the middle of the fields. Usually, streak is limited to a few plants at the edge of the field or to scattered plants in the field. The worst outbreaks of the disease have been reported from Boone, Pendleton, Grant, and neighboring counties in the extreme northern part of the State, but it is generally state-wide.

The virus of streak is transmitted with difficulty mechanically, if at all, unless very recently invaded necrotic tissue is used as inoculum<sup>5</sup>; and even then the percentage of positive transfers may be low.

Tobacco usually does not live through the winter in Kentucky, and the virus does not appear to be seed transmitted; therefore, it seems obvious that it has some other host, which is at least biennial, and that an insect vector must be concerned.

## SWEETCLOVER A PROBABLE SOURCE OF STREAK VIRUS

Sweetclover (*Melilotus alba*) is affected by a virus that we have been unable to transmit mechanically to tobacco. It is characterized by chlorosis and sometimes chloritic or necrotic ring and line patterns. The virus is commonly observed in small patches of sweetclover and in sweetclover that is pastured or mowed, but in extensive undisturbed plantings the disease usually is not evident. Observations of outbreaks of streak in

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>2</sup> Valneau, W. D., and E. M. Johnson, Tobacco Diseases in Kentucky. Kentucky Agr. Exp. Stat. Bull. 328: 111-154. 1932.

<sup>3</sup> Unpublished.

<sup>4</sup> Johnson, J., Tobacco streak, a virus disease. *Phytopath.* 26: 285-292. 1936.

<sup>5</sup> This suggestion was obtained from J. Johnson, who used the method in his studies of the streak virus.

Pendleton County, where sweetclover grows along roadsides and in waste areas and is used as a soil-building crop, suggested to the writer in 1937 that there was a relation between outbreaks of streak in tobacco and sweetclover, as, near each field where streak was severe, sweetclover was found. Many observations since that time in northern, central, and western Kentucky have indicated that where streak occurs sweetclover may be expected to be found in the immediate vicinity. Sweetclover is not a common weed in Kentucky, except in the northern part of the State where limestone outcrops. In other parts of the State it is sometimes a roadside weed. Several specimens of streak in Burley tobacco have been received from Burley-tobacco growers in Missouri, and, in each case, inquiry revealed that sweetclover was growing adjacent to the affected plants.

Following the writer's visit to Pendleton County on July 20, 1937, C. E. Bortner called his attention to a severe outbreak of streak in Burley tobacco in a recently established tobacco rotation on the Experiment Station farm at Lexington, where tobacco was growing for the first time in a plot adjacent to 2nd-year sweetclover. Whereas in previous years not over 3 or 4 streak plants had been found any year on the farm in about 50,000 plants, this small plot (150 plants) had 13 streak plants on that date and, in the following 2 years, streak was abundant in comparable plots (Table 1). In 1939, .12 per cent streak developed in the remainder of the rotation series, but 10 of the 15 streaked plants were within a short distance of sweetclover plots.

TABLE 1.—*Streak in Burley tobacco growing adjacent to second-year sweetclover*

Year	Plot no.	No. of streak plants	Percentage streak in plots	Percentage streak in other plots
1936 <sup>a</sup>		0	0.0 <sup>b</sup>	
1937	529	25	16.6	.035
1938	629	32	21.3	
1939	729	33	22.0	.12 in 13,650 plants

<sup>a</sup> No second-year sweetclover was present in the entire series.

<sup>b</sup> Counts were made by C. E. Bortner.

The relatively high percentages of streak for each of 3 years, in tobacco growing within 20 feet of sweetclover plants, and the extremely low percentage in the remainder of the series and on other parts of the farm year after year give nearly conclusive proof that the virus was transmitted from the sweetclover. The weed population in the sweetclover would certainly be duplicated on other parts of the farm. It also suggests that the insect vector travels relatively short distances, or, if it travels longer distances, seeks other host plants than tobacco. Infection in tobacco occurs at about the time sweetclover seeds are forming, suggesting that, because of the hardening of sweetclover, the vector is forced to seek other food plants. It is

unlikely that tobacco is one of the preferred hosts of the vector, so that infection on tobacco is probably purely accidental.

That the growing of sweetclover in extensive plantings is not necessarily a menace to tobacco growing in the vicinity is indicated by the following observations: A tobacco grower in Woodford County has, for 18 years, grown tobacco following a rotation of winter wheat, sweetclover, and weeds for about 3 to 5 years. He does not disturb the sweetclover in any way, such as by cutting or pasturing, until it is plowed for tobacco. There are both one- and two-year sweetclover in these fields, as the ground contains quantities of seed. The rotation is excellent because the value of the Burley crop has increased each rotation during this period. Although tobacco is frequently grown near sweetclover, streak plants are extremely rare on two farms handled in this manner, but an occasional affected plant has been found. The sweetclover appears to be relatively free from virus disease. The supposition is that the insect vector, not being disturbed by stock pasturing in the sweetclover, or by mowing, does not move great distances. Consequently, the virus is not spread rapidly and the majority of vectors remain nonviruliferous. When the old sweetclover dies there is an abundance of young plants on which to feed.

In contrast a field of tobacco in the same county was observed a part of which was growing next to a railroad where scattered plants of sweetclover were growing. The grower insisted that the sweetclover be mowed. About 2 weeks later, 29 per cent of the tobacco plants in several rows parallel to the railroad were affected with streak, while the tobacco in the same planting about 50 yards away had only scattering streak plants. Evidently either the mowing or maturity of the sweetclover made it necessary that the vector move and it had migrated into the nearby tobacco, fed, and transmitted the virus.

#### CONCLUSIONS

These observations, while not conclusive, indicate that sweetclover is probably the host from which an insect vector carries the streak virus to tobacco growing in the vicinity. Roadside plants or scattered sweetclover plants in waste places seem more likely to become infected, and to act as sources from which tobacco may become infected, than extensive sweetclover plantings if these are not disturbed by grazing or mowing. Sweetclover sowed in fields not well stocked with sweetclover seed might be a source of infection of nearby tobacco because of migration when the sweetclover became tough during ripening. The presence of young clover might prevent migration. It is probable that the destruction of second-year sweetclover in the immediate vicinity of fields to be planted to tobacco, before the tobacco is planted, will result in control in areas where the disease has proved injurious.

# DIAPORTHE VACCINII, THE ASCIGEROUS STAGE OF PHOMOPSIS, CAUSING A TWIG BLIGHT OF BLUEBERRY

MARGUERITE S. WILCOX

(Accepted for publication January 8, 1940)

## INTRODUCTION

In 1936 the writer reported the occurrence of a Phomopsis twig blight on the cultivated blueberry, *Vaccinium corymbosum* L., in both Massachusetts and New Jersey.<sup>1</sup> In a later paper<sup>2</sup> this blueberry twig-blight fungus was shown to be identical with *Phomopsis vaccinii*, the imperfect stage of *Diaporthe vaccinii* Shear, which causes a serious decay in the fruits of the cranberry, *Vaccinium macrocarpon* Ait. The present paper reports the development of the perithecial stage in cultures of the blueberry twig-blight *Phomopsis* and its identity with *Diaporthe vaccinii*.<sup>3</sup>

## SOURCE OF CULTURES

Cultures in which perithecia developed were obtained from the following 3 sources: Subcultures from original isolations made in 1936 from small sterilized pieces of naturally blighted blueberry shoots; the pulp of decayed cranberry fruit; and reisolations of the *Phomopsis* from diseased areas of artificially infected blueberry plants. Cultures were made on cornmeal agar in July, 1938; and, in October, while producing only pycnidia, 17 were placed out of doors at the United State Horticultural Station, Beltsville, Maryland, and not again examined until February, 1939.

All the cultures were then found to have occasional small, thick, stroma-like bodies, black on the exterior, partly or wholly embedded in the substratum (Fig. 1, C, a and b), in which were *Phomopsis* pycnidia and perithecia of the *Diaporthe* type, either together in the same stroma (Fig. 1, B, a and b) or in separate stromata (Fig. 1, A). The occasional formation of pycnidia and perithecia in the same stroma is a cultural characteristic of cranberry isolates of *Diaporthe vaccinii*. Perithecia-bearing stromata continued to form for several months in these cultures, kept in a refrigerator at a temperature of 8° C. Single-ascus transfers from cultures made originally from naturally blighted blueberry shoots later produced the ascigerous stage at room temperature.

In the original description<sup>4</sup> of *Diaporthe vaccinii*, Shear<sup>3</sup> states that

<sup>1</sup> Wilcox, Marguerite S. Notes on blueberry fungi. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 20: 106-107. 1936.

<sup>2</sup> Wilcox, Marguerite S. Phomopsis twig blight of blueberry. Phytopath. 29: 136-142. 1939.

<sup>3</sup> Shear, C. L., N. E. Stevens, and H. F. Bain. Fungous diseases of the cultivated cranberry. U. S. Dept. Agr. Tech. Bull. 258. 1931.

<sup>4</sup> "In stromata on cranberry fruit and in culture, but separate and without any trace of stroma on dead cranberry vines; perithecia on stems grow between bark and wood, with eccentric neck protruding through the bark, nearly hemispherical, 0.3-0.5 by 0.2-0.4 mm.; wall two to several cell layers thick, black, carbonous; on decayed berries perithecia in stromata, with long perithecial necks protruding in all directions from folds of the shriveled berry; in cornmeal agar cultures perithecial stromata are partly embedded, about 1.5-2 mm."

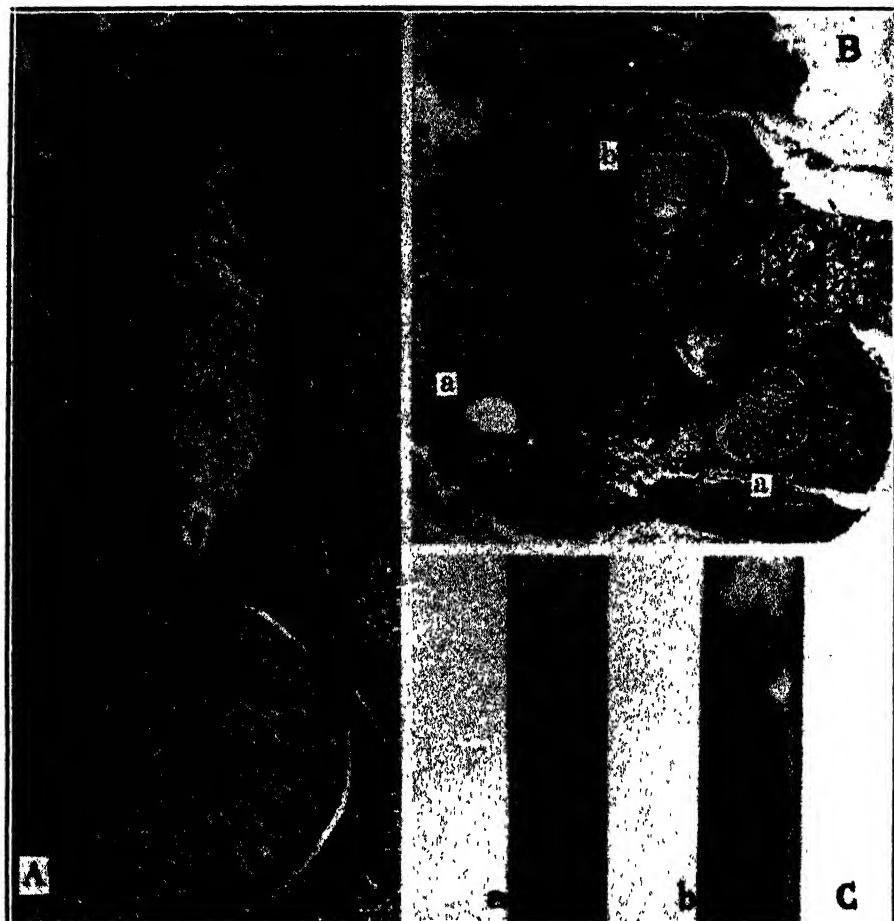


FIG. 1. *Diaporthe vaccinii* in cornmeal-agar cultures. A. Perithecioid in stroma embedded in agar. Isolate from naturally blighted blueberry shoot.  $\times 135$ . B. Cross section of stroma embedded in agar. Reisolated from artificially infected blueberry plant. a. Perithecia. b. Pycnidia.  $\times 56$ . C. Cultures of *Diaporthe vaccinii*. a. From naturally blighted blueberry shoots. b. From pulp of decayed cranberry fruits.  $\times 3$ .

the perithecia are formed in stromata on cranberry fruits and in culture, but separate and without any trace of stromata on dead cranberry vines. The ascigerous stage of *Phomopsis vaccinii* has not been found on the blueberry in nature; consequently, it is not known whether the perithecia should be expected to occur separately (not in stromata), as is the case on cranberry.

In cornmeal-agar cultures of the blueberry isolates, the perithecia are definitely stromatic, and both stromata and perithecia appear to be iden-

in diameter, with numerous beaks growing to a length of 0.5 mm.; perithecial necks several cells thick, heavy-walled, black, carbonous, copiously supplied with upward-directed hairs; asci oblong fusoid, sessile, 37–51 by 6.8–11.7  $\mu$ , apex thickened and pierced by a narrow pore; spores irregularly biserrate, ellipsoid, obtuse; 2-celled, slightly constricted at the septum, each cell typically biguttulate, 8.8–11.8 by 2.4–3.4  $\mu$ .'' (p. 7)

tical with the ascigerous stage of the cranberry *Diaporthe*, as described by Shear, from cultures.<sup>5</sup> The production of stromata containing both pycnidia and perithecia (Fig. 1, B, a and b) and the elongated thick-walled ostioles copiously supplied with upward-directed hairs (Fig. 1, A) are additional evidence of the identity of the isolates from the two hosts. The asci are oblong, fusoid, sessile with narrow pores, 32–48 by 5.8–9.6  $\mu$ ; spores 2-celled, slightly constricted, and biguttulate, 6.4–12.8 by 2.5–4.2  $\mu$  (Fig. 2).

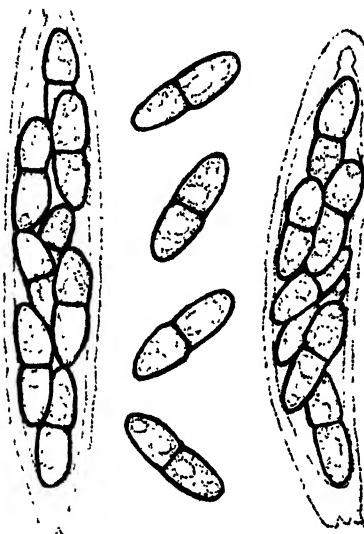


FIG. 2. *Diaporthe vaccinii*. Asci and ascospores from blueberry isolate grown on cornmeal agar.  $\times 1360$ .

At the same time comparative measurements were made of *Diaporthe vaccinii* from cultures made from the pulp of decayed cranberry fruits. The asci were 35–45 by 6.4–9  $\mu$ , and the spores 6.4–11.6 by 3.2–3.8  $\mu$ . The asci and spores, the formation of perithecia in stromata, and all cultural characteristics of *Diaporthe* from the blueberry isolates appear identical in every respect with *Diaporthe vaccinii* Shear,<sup>5</sup> the cause of a decay in cranberry fruits.

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<sup>5</sup> See footnote 3

# THE RELATIONSHIP BETWEEN VIRUSES OF POTATO CALICO AND ALFALFA MOSAIC

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(Accepted for publication Dec. 5, 1939)

It was observed that potato-calico virus produces symptoms in *Nicotiana glutinosa* L. very similar to those caused by alfalfa-mosaic virus (*Marmor medicaginis* H.)<sup>1</sup> in the same host plant. This observation suggested that the two viruses might be closely related. It is the purpose of this paper to point out the similarities and differences in the symptoms of the two diseases and to report the results of cross protection tests. The investigation showed that the two viruses, although distinct, are indeed closely related.

## VIRUS STOCKS

Potato-calico virus was obtained from E. S. Schultz. It is apparently identical with that studied by Porter (5, 6, 7) and Dykstra (2). On the basis of symptomatology, Porter (6) distinguished between the viruses of potato-calico and potato-aucuba mosaic (*Marmor aucuba* H.). Dykstra (2) likewise distinguished between these viruses and reported further that potato-calico virus is apparently unrelated to the viruses of potato-aucuba mosaic and Canada-streak (*M. aucuba* H. var. *canadense*, n. var.),<sup>2</sup> a conclusion which is confirmed in the present paper.

Alfalfa-mosaic virus was secured from H. T. Osborn. It is believed to be the same as or a closely related strain of that studied by Zaumeyer and Wade (10) and Zaumeyer (11) and designated by the latter as alfalfa-mosaic virus 1. The literature is somewhat confused as to whether there are one or two alfalfa-mosaic viruses. Pierce (4) distinguished between the alfalfa-mosaic virus studied by Weimer (8, 9) and that studied by himself and accordingly referred to the viruses as alfalfa viruses 1 and 2, respectively. Zaumeyer (11), on the other hand, considered Weimer's virus and Pierce's virus to be the same and referred both to alfalfa-mosaic virus 1. The writers agree that there is insufficient evidence for distinguishing between alfalfa viruses 1 and 2.

## COMPARATIVE SYMPTOMATOLOGY

Both potato-calico virus and alfalfa-mosaic virus were transmitted to several species of plants by the rubbing method of inoculation. In most cases earborundum was employed. Both viruses were readily transferred from young *Nicotiana glutinosa* plants to other plants of the same species. They produced almost identical symptoms after an incubation period of 3 to 4 days.

<sup>1</sup> Latin binomials used in this paper are based on the system of nomenclature in the Handbook of Phytopathogenic Viruses (3).

<sup>2</sup> It has been shown (1, 2) that the viruses of potato-aucuba mosaic and Canada-streak are related. The comparative symptomatology of the induced diseases clearly indicates that the viruses are not identical. For this reason, it is felt that the Canada-streak strain of virus should have varietal rank. The name *canadense*, suggested by the common name, seems appropriate.

*N. glutinosa* proved to be a useful test plant and a good source plant for both viruses. On beans (*Phaseolus vulgaris* L. var. Early Golden Cluster and Corbett Refugee) and on Black Eye cowpea (*Vigna sinensis* Endl.) they produced the same type of necrotic primary lesion. On beans, lesions sometimes appeared within 24 hours after inoculation. Both viruses produced necrotic primary lesions followed by a systemic streak disease in broad bean (*Vicia faba* L.). On seedlings of Green Mountain potatoes (*Solanum tuberosum* L.), they produced similar symptoms but the symptoms of potato-calico virus were more severe than those caused by alfalfa-mosaic virus. Both viruses caused mottling and necrotic vein-banding in crimson clover (*Trifolium incarnatum* L.) and in red clover (*T. pratense* L.). Both produced a mottling disease in white clover (*T. repens* L.), and bright yellow spotting in leaves of the Improved Long Green cucumber (*Cucumis sativus* L.). The similarity in the rather distinctive reactions of the 2 viruses in these hosts strongly suggested that they might represent strains of one virus.

That potato-calico and alfalfa-mosaic viruses are not identical is shown by the fact that the former is the milder of the two in *Nicotiana glutinosa*, crimson clover, and red clover, whereas the latter is the milder in potato. Moreover, under comparable conditions, the potato-calico virus produces fewer lesions in kidney beans, broad beans and cowpeas than does alfalfa-mosaic virus.

#### CROSS PROTECTION TESTS

Cross-inoculation experiments were made on *Nicotiana glutinosa* and *N. tabacum* L. var. Turkish. It should be pointed out that in *N. glutinosa* both viruses cause diseases showing an acute stage with severe symptoms followed by a chronic stage with mild symptoms. Groups of six young *N. glutinosa* plants were inoculated with one or another of the viruses causing the following diseases: Alfalfa-mosaic, potato-calico, potato-ringspot (caused by *Marmor dubium* H. var. *annulus* H.), cucumber-mosaic (caused by *M. cucumeris* H. var. *rulgare* H.), and Canada-streak. Juice from healthy *N. glutinosa* plants was rubbed over the leaves of six additional plants. Twelve days later, when the inoculated plants were systemically infected, three upper leaves on each of three plants in each group were inoculated with juice from *N. glutinosa* plants infected with alfalfa-mosaic virus. Leaves on the remaining three control plants in each group were similarly rubbed with juice from healthy *N. glutinosa* plants. The plants were observed for two weeks. None of the control plants rubbed with juice from healthy plants developed additional symptoms. Of the leaves rubbed with juice containing alfalfa-mosaic virus, those previously infected by potato-calico virus or alfalfa-mosaic virus were alive and green at the end of this period; the others were dead or moribund. Moreover, the new leaves on the plants previously infected with potato-calico or alfalfa-mosaic showed no symptoms in addition to those characteristic of the chronic stages of these diseases while new leaves on the other plants developed systemic necrotic lesions typical of the acute stage of alfalfa-mosaic.

Similar results were obtained when plants of *Nicotiana tabacum* var. Turkish that had previously been infected with the potato-calico virus were inoculated with alfalfa-mosaic virus. The inoculated leaves of these plants developed no necrotic primary lesions, whereas leaves of previously healthy

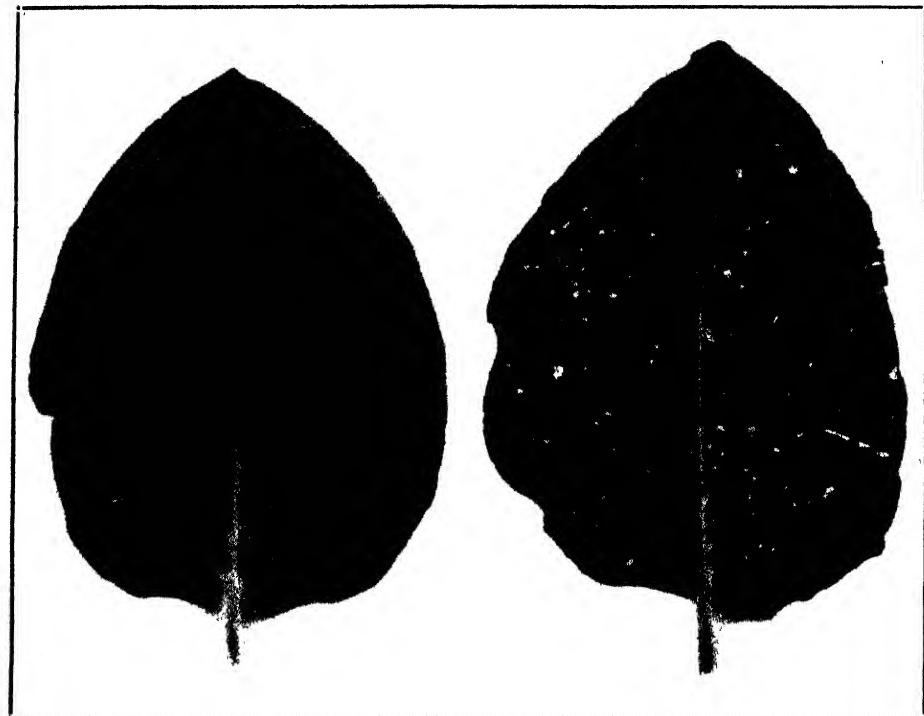


FIG. 1. Leaves from a cross inoculation test on *Nicotiana tabacum* var. Turkish. Both leaves were inoculated with alfalfa-mosaic virus. The leaf on the right had previously been healthy, that on the left had been invaded by the virus of potato calico. The symptoms of potato calico were mild and do not show in the photograph. (Photograph by J. A. Carlile.)

plants (Fig. 1) or plants infected with cucumber-mosaic virus developed many such lesions.

The protection described above is considered good evidence that potato-calico and alfalfa-mosaic viruses are closely related. The potato-calico virus is, therefore, classified as a strain of *Marmor medicaginis* and given the varietal name *solani* from NL. *Solanum*, generic name for the potato. Alfalfa-mosaic virus should be designated as *M. medicaginis* H. var. *typicum* n. var. to distinguish it from the potato-calico strain of the virus.

#### SUMMARY

Potato-calico virus and alfalfa-mosaic virus produce similar but not identical symptoms on *Nicotiana glutinosa* L., *Phaseolus vulgaris* L., *Vicia faba* L., *Vigna sinensis* Endl., *Solanum tuberosum* L., *Trifolium incarnatum* L., *T. pratense* L., *T. repens* L., and *Cucumis sativus* L.

Plants of *Nicotiana glutinosa* and *N. tabacum* infected with potato-calico virus are refractory to infection with alfalfa-mosaic virus. Potato-calico and alfalfa-mosaic viruses are, therefore, considered to be closely related and the potato-calico strain is named *Marmor medicaginis* H. var. *solani* n. var. Plants affected by potato-ringspot, cucumber-mosaic or Canada-streak are susceptible to infection with alfalfa-mosaic virus. Therefore, the viruses causing these diseases are not thought to be closely related to alfalfa-mosaic virus.

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#### AN INCUBATING CAN FOR LABORATORY OR FIELD USE

FREEMAN WEISS AND FLOYD F. SMITH

(Accepted for publication Dec. 4, 1939)

The advantages of glass for many kinds of laboratory receptacles must sometimes be sacrificed for materials of greater durability in work carried on in temporary laboratories or in the field, and initial cost, as well as replacement costs, may also become important. Metal receptacles have not been widely used in place of glass because of the unavailability of a range of sizes and forms, the tendency of ordinary tinned iron vessels to corrode, and the expense of noncorrosive metals. Improvement in lacquering processes is now overcoming some of these difficulties.

In connection with investigations of azalea flower spot (*Ovulinia azaleae* Weiss) carried on in an improvised field laboratory at Magnolia Garden, near Charleston, South Carolina, from 1936 to 1939, we had occasion to keep a large number of flowers under observation for periods of several days up to 2 weeks. It was essential that each sample be segregated to prevent accidental infection, and it was desirable that all samples be kept under approximately uniform conditions of temperature and atmospheric humidity. Celuloid cages were first tried for this purpose in the form of a cylinder about

10 inches high and 5 inches in diameter mounted on a wooden base, which inclosed the neck of a 6- or 8-ounce bottle. For our purpose these proved too bulky, expensive, and difficult to clean and they had the further defect of contributing some toxic emanation to the atmosphere within the cage so that a very low proportion of infection was obtained.

Tin-plated 1-lb. coffee cans were next tried. A 2-dram homeopathic vial was fastened to the inside next the bottom, using either a strip of adhesive tape or a wire passing through the wall with the ends twisted together outside. The vial was buttressed with high-melting-point paraffin so as to form an even slope from its mouth to the bottom of the can and thus eliminate crevices at its base, which would be difficult to clean. In use, the vial was filled with water from a rubber syringe, and an azalea twig bearing 1 to 4, usually 2, flowers was inserted. A piece of paper towel was placed on the bottom of the can to take up any excess water. The flowers were exposed to insect contact or inoculated in various ways, and were usually atomized with water before the cans were covered and set away. Infection by *Orulinia* was readily obtained when only a small drop of a spore suspension in water was placed with a pipette on each petal, without atomizing. Such drops usually persisted, without evaporating, for at least 24 hours. Freshly cut azalea flowers remained in good condition in these cans for 8 to 12 days, which was more than ample for the required observations. About 200 of these cans could be stacked in an improvised thermostatic case, approximately 40 inches in each dimension. The consistency and uniformity of infection obtained in replicate samples throughout the case showed that the

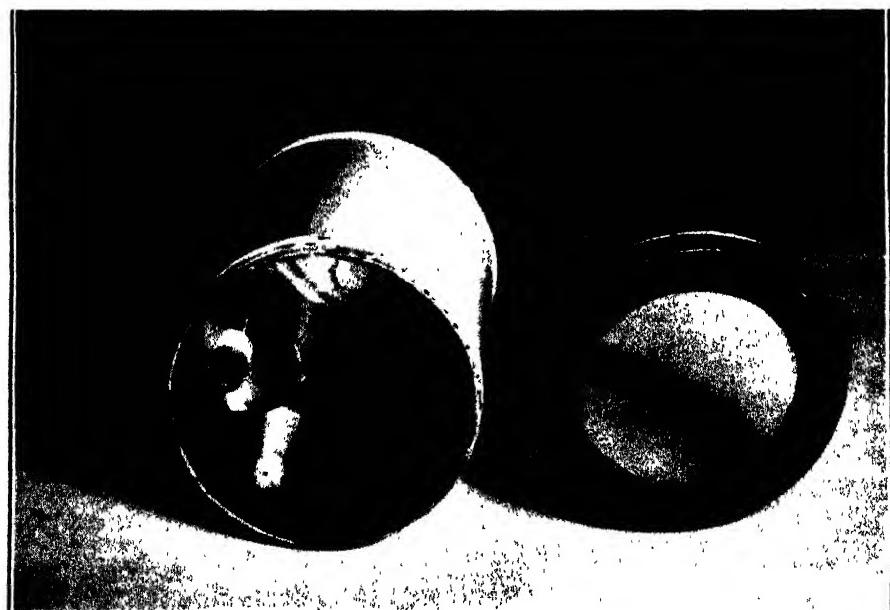


FIG. 1. Incubating can with vial as water reservoir and metal ring and celluloid disc as cover.

requirements of homogeneous moisture and temperature conditions were well met.

An improvement in this type of can was found in a lacquered can intended for the sale of a corn chip or cracker. This can was 4 in. in diameter and 4½ in. high (Fig. 1). The lacquer prevented rusting, and the top, instead of depending on friction, was provided with a grip lock, easily removable yet capable of firm attachment. These cans could be carried, in cartons of 24, into the field on distant collecting trips; they were light, unbreakable, and not easily upset.

These "incubating cans" have been used for experimental inoculations with other fungi, including *Cladosporium paoniae*, *Puccinia malvacearum*, etc. Small cut shoots and stalked leaves keep in good condition in them up to 2 weeks or longer. Suitable atmospheric humidity for infection seems to be more uniformly maintained in this type of vessel than in the usual moist chamber or bell jar, because of the tight cover and the relatively small surface on which condensation of moisture can occur.

The cans can be satisfactorily cleaned for most purposes by thorough washing with soap and water, but if one wants to dispense with the paraffin buttress around the vial, they may be sterilized in an autoclave or a drying oven. Exposure to 15 pounds of steam pressure did not visibly affect the lacquer, and heating at 243° C. for 2½ hours in a drying oven only darkened it.

For experimental inoculations influenced by light, the top of the can may be cut out on a lathe or with a circular metal cutter to form a ring, and a disc of celluloid inserted between the can and the cover.

The cost of these cans, when obtained from the manufacturer in quantities of 5000, is approximately 4½ cents each, as compared with 25 cents to \$1.00 for Petri dishes and glass "moist chambers."

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#### PHYTOPATHOLOGICAL NOTES

*Losses from Bunt of Wheat in the United States.*—The difficulty of estimating crop losses caused by disease is admittedly so great that there exists some skepticism as to the validity of the published estimates. It is apparent then that any evidence making it possible to check some of these estimates against another method of measuring disease losses, is worth consideration. In figure 1, A, are presented the estimated losses from bunt of wheat in the United States, as compiled from reports from collaborators of the Plant Disease Survey, and the percentage of all ears grading smutty, as indicated by reports of federal grain inspectors. The accuracy of these reports of federal inspection and their usefulness as a source of plant-

disease information have been commented on elsewhere,<sup>1</sup> and Haskell and Boerner<sup>2</sup> have noted specifically the correlation between the amount of bunt in the field and the smuttiness of threshed grain.

The figures for percentage of all cars grading smutty have been com-

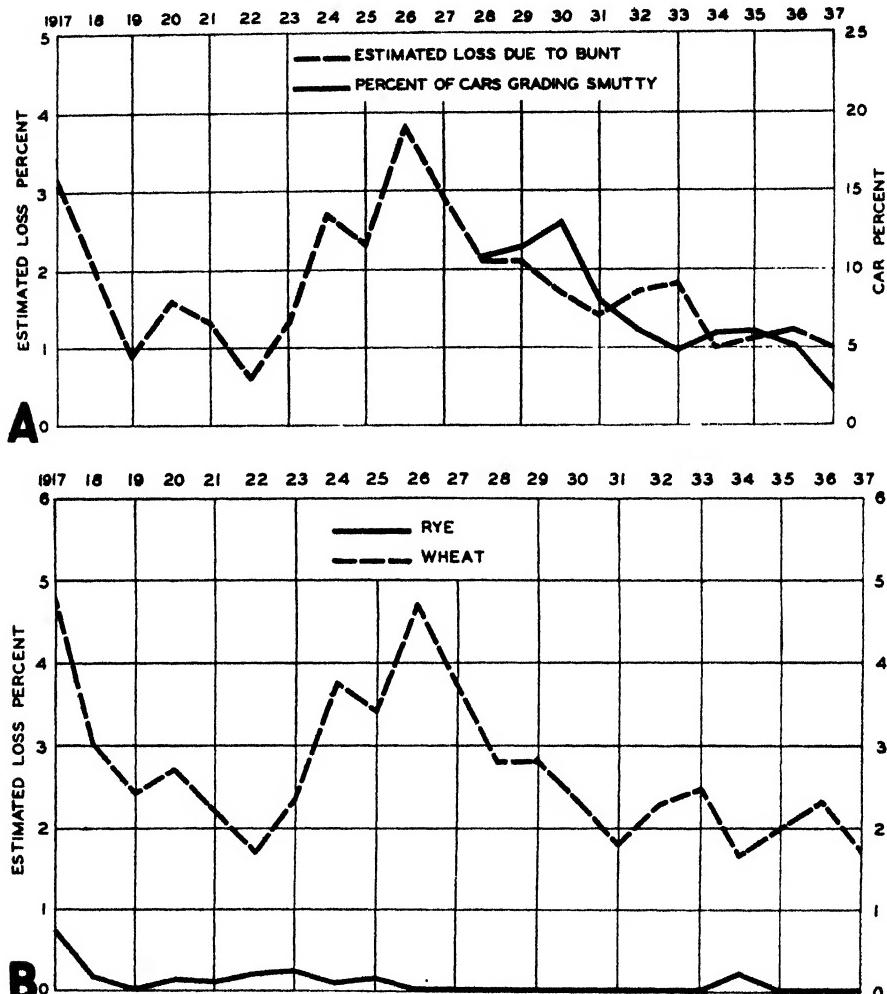


FIG. 1. A. Estimated losses from bunt of wheat in United States (reporting area) 1917-1937 and percentage of cars grading smutty at all terminals, 1928-1937. B. Estimated losses from smuts of wheat and rye in United States (reporting area) 1917-1937, compiled from reports from collaborators of the Plant Disease Survey.

puted for all the different kinds of wheat, beginning with the season of 1928-1929, and were given to the writer by Fred G. Smith, Chairman of the Educational Committee of the Office of Federal Grain Supervision. From

<sup>1</sup> Stevens, N. E. Incidence of ear rots in the 1916-1933 corn crops. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 19: 71-93. 1935.

<sup>2</sup> Haskell, R. J., and E. G. Boerner. Relation of stinking smut of wheat in the field to smuttiness of threshed grain. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. Sup. 79: 1-5. 1931.

these we have computed the figures for the United States as a whole. When compared with the estimated losses in wheat caused by bunt, it is evident that the two show, in general, the same trend—a decline in the abundance of bunt in the United States during recent years. When it is remembered from what wholly different sources these figures are derived, and that a number of States are not included in the crop-loss estimates, the agreement certainly appears significant.

The same sources of information afford a means of checking the relative smut-induced losses in wheat and in rye. Among the "special grades" the definitions of "smutty" are identical for wheat and for rye,<sup>3</sup> and smutty ears of rye are occasionally reported. The number of ears of rye grading smutty during the decade for which figures are available has, however, been too small to be recorded on the graph. This difference in the disease relations of the two crops agrees with the reports of the collaborators of the Plant Disease Survey (Fig. 1, B).

Comparison of A and B of figure 1 shows how large a part of the total estimated loss from smuts in wheat in the United States during this period was due to bunt.—NEIL E. STEVENS, University of Illinois, Urbana, Illinois.

*Coniothyrium fuckelii* Sacc. on Rose Leaves.—Young leaves of rose, variety Joanna Hill, which had been inoculated with *Diplocarpon rosae* and kept floating on sucrose solution for 7 days, revealed pycnidia of some other fungus within the black-spot lesions. This fungus was identified as *Coniothyrium fuckelii* Sacc. It was isolated, and inoculated into rose canes

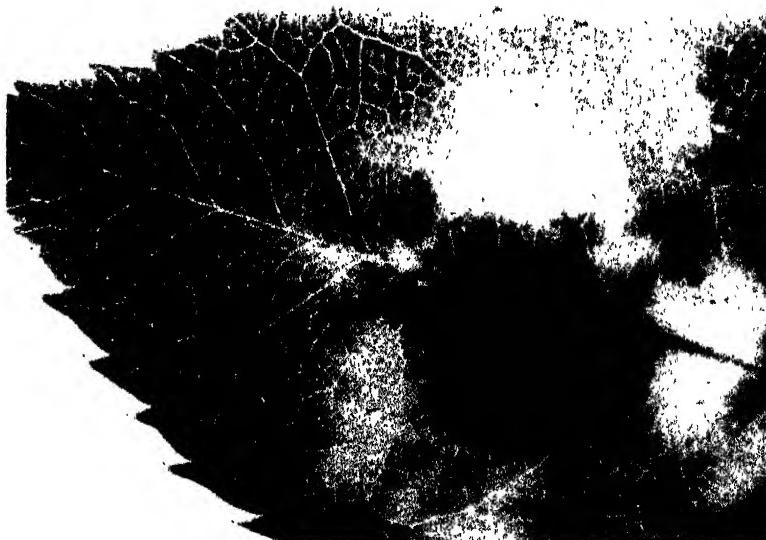


FIG. 1. Pycnidia of *Coniothyrium fuckelii* within black-spot lesion caused by *Diplocarpon rosae*. Photographed two weeks after inoculation.  $\times 4\frac{1}{2}$ .

<sup>3</sup> Handbook of Official Grain Standards of the United States. U.S.G.S.A.—Form No. 90, Revised June, 1937.

where it caused lesions typical for those of the stem and graft canker of roses.

Uninjured young leaves of rose shoots of the varieties Joanna Hill and Talisman, when inoculated with a spore suspension of this fungus and kept for 2 weeks under favorable conditions of temperature and humidity, remained healthy; whereas leaves inoculated with a mixture of spores of *Coniothyrium fuckelii* and *Diplocarpon rosae* bore pyenidia of the former fungus intermingled with acervuli of the latter within the black-spot lesions. Pyenidia were found both on the upper and the under leaf surfaces, and bore numerous spores.

It thus seems that *Coniothyrium fuckelii*, the causal organism of the stem and graft canker of roses, is able to fruit on rose leaves attacked by *Diplocarpon rosae*; whether it is able to follow other pathogens of the rose foliage, has not been determined.

Since close inspection is necessary in order to distinguish the fruit bodies of the two fungi under discussion, the occurrence of *Coniothyrium fuckelii* on black-spotted rose leaves might have escaped attention, although nothing is known yet as to what extent that fungus does follow black spot under greenhouse conditions.—KARLA LONGRÉE, Department of Plant Pathology, College of Agriculture, Cornell University, Ithaca, N. Y.

*Heterothallism in Venturia pirina*.—The 8 spores of each of 5 asci of *Venturia pirina* Aderhold were isolated in the order of their occurrence in the ascus and grown *in vitro*. Petri dishes containing 20 cc. each of 0.5 per cent malt-extract (Trommer's) agar plus a decoction of dead pear leaves were seeded with conidia from these isolates, used singly and in every possible pairing within each set of 8. Similar seedlings were made on sterilized dead pear leaves in test tubes containing a few cc. of malt-extract decoction. The cultures were incubated 4 months at temperatures favorable for the development of perithecia.

All the isolates proved to be hermaphroditic and self-sterile. Each set is comprised of 2 groups of 4 isolates each, which are intra-group sterile and inter-group fertile. Segregation for sexual compatibility occurred alternatively in the first or the second nuclear division in the ascus. Pairings of the isolates between certain sets revealed only 2 compatibility groups.—M. H. LANGFORD and G. W. KEITT, University of Wisconsin, Madison, Wisconsin.

*A Preliminary Report on Variability and Inheritance in Venturia inaequalis*.—The 8 ascospores of each of 4 asci were isolated in the order of their occurrence in the ascus and grown on malt agar through 12 successive monoconidial transfers made at approximately 8-week intervals. The thalli showed distinctive morphological characters, which were comparatively constant in culture. Each set of 8 isolates contained 4 pairs that behaved alike, indicating that the third nuclear division in the ascus is equational.

The pathogenicity of all cultures was studied 2 seasons on 9 apple varieties. Differential varieties were found for all sets. Pathogenicity, under approximately the same environmental conditions, remained comparatively constant.

A method was developed for growing the ascigerous stage of the fungus *in vitro*. The 32 isolates of the 4 sets fell into 2 groups of 16 each for sexual compatibility, being self-sterile, intra-group sterile, and inter-group fertile. Segregation for sexual compatibility occurred alternatively in the first or second nuclear division in the ascus.

Occasional sectors appeared in culture. They grew faster, produced fewer conidia, and were less pathogenic than their parent isolates. One incited white lesions on leaves. In several cases a sector and its parent isolate were separately paired *in vitro* with the same compatible isolate. The ascocarps from pairings involving the parent isolates had 8-spore asci. Those from 2 pairings involving sectors had 4-spore asci, indicating that these sectors differed from their parent isolates in genetic constitution.—  
G. W. KEITT and M. H. LANGFORD, University of Wisconsin, Madison, Wisconsin.

*A Note on the Status of the Generic Name Urocystis.*—In the interest of stability in nomenclature, present trends in mycology and phytopathology are definitely toward adherence to the present International Rules of Botanical Nomenclature. Strict interpretation of these rules, however, would necessitate a number of changes in well-known generic names, established by usage over many years. To obviate undesirable changes of this character provision was made, at the International Botanical Congresses at Cambridge and Amsterdam, for a special committee on fungi authorized to consider the matter of *genera conservanda*. The report of this committee, including a list of generic names proposed for conservation, is to be presented to the next Congress. Even though it is not yet known when the next Congress will convene, it seems desirable to continue the work.

Of the various genera involved, *Urocystis* has been of particular interest, and has been included in the list of proposed *nomina generica conservanda* published by the Nomenclature Committee of the British Mycological Society.<sup>1</sup> The Plant Pathological Committee of the same Society also has endorsed this proposal.<sup>2</sup> As pointed out in the above reference, the name *Tuburcinia* is the legal name under the provisions of the International Rules, but the following reasons are advanced for conserving *Urocystis* Rabenh. (1856) against *Tuburcinia* (1832).

"(1) The name *Urocystis* has been well known to, and in frequent use by, plant pathologists since there has been a science of plant pathology, and should, accordingly, not be discarded without cogent reasons.

"(2) The disuse of the name *Urocystis* is not dictated by the accession of any new knowledge. It has been known and accepted that *Tuburcinia*

<sup>1</sup> Trans. Brit. Myc. Soc. 23: 223. 1939.

<sup>2</sup> Loc. cit. 231. 1939.

*Orobanches* is a species of *Urocystis* since 1877, when it was renamed *Urocystis Orobanches*. For more than forty years mycologists refrained from transferring the species of *Urocystis*, *en bloc*, to *Tuburcinia*, so as not to confuse the literature.

"(3) Since 1887, the generic name *Tuburcinia* Fr. has been used in a rather different sense, as if it were founded on *T. Trientalis* Berk. and Br., a species unknown to Fries. It is still a matter of taxonomic dispute whether species of *Tuburcinia* so used (and none of them are major pathogens) are properly classified in the same genus with the species of the genus *Urocystis*."

For the above reasons the present committee recommends the continued use of the generic name *Urocystis* until such time as an International Botanical Congress shall have definitely passed on the proposal to conserve it.—Committee on Fungus Nomenclature, G. L. ZUNDEL, Chairman, J. A. STEVENSON, C. M. TUCKER, D. S. WELCH, AND ERDMAN WEST.

### BOOK REVIEWS

MOORE, W. C. *Diseases of Bulbs*. Bull. 117, Min. Agric. and Fish. Gt. Britain. 176 pp., 58 figs. 1939. Publ. by H. M. Stationery Office, London. Pr. 4 s. U. S. Office, Brit. Libr. Information, 270 Madison Ave., New York. Pr. \$1.20.

This publication should go far to dispel any connotation of mediocrity that often attaches to government agricultural bulletins. It is immediately noteworthy as an example of skilful and artistic presswork, with fine quality of paper and clear type. The 58 half-tone illustrations are superlative in both photographic excellence and clear reproduction. The value of the text for general information and as a reference work is even more appealing to one who is familiar with the subject matter. It covers the diseases of all the major bulbous crops of the Amaryllidaceae, Iridaceae, and Liliaceae and includes those of such relatively unknown (pathologically) bulbous plants as *Colchicum*, *Convallaria*, *Muscari*, *Ornithogalum*, *Scilla*, and others. For the parasitic diseases the treatment includes not only the customary descriptive and historical material, and an authoritative discussion of control, but also a complete mycological account with authentic information on synonymy, morphology, and life history of the causative organisms. The latter feature should especially commend itself to American students of the pathology of ornamentals, since much of the earlier mycological work on bulbs was published in garden and nursery periodicals, which were not adequately indexed in this country. Those written in the Dutch language have remained largely unknown here for want of competent translations. There are 709 literature citations! Practical bulb growers and students will find nothing in the English language to compare with it.—FREEMAN WEISS.

COOK, MELVILLE THURSTON. *Enfermedades de las plantas economicas de las Antillas*. (Translated from the original manuscript by José I. Otero.) Monograph of the University of Puerto Rico (Rio Piedras) Ser. B. (Physical and Biological Sciences), No. 4. 530 pp., frontispiece, 171 figs. 1939. \$2.00.

Although there have been numerous workers in the field of phytopathology throughout that vast area included in Spanish-speaking America, with many and important contributions to the science, no general work on the subject has appeared in the Spanish language for this region until the publication of the present volume.

Pertinent papers have been published quite commonly in the United States or Europe, usually in languages other than Spanish, or they have appeared in local agricultural journals of limited distribution or in more or less ephemeral bulletins and reports that are relatively unavailable to those in need of the information.

The present volume becomes the first Spanish plant pathology to be published in the New World. In point of present-day usefulness it is the only such text available from any source. Gonzalez-Fragoso, it is true, issued his *Botanica Criptogamica Agricola* in 1927, but it is very definitely adapted to the crops and conditions prevailing in Spain, has been difficult to obtain, and is now presumably no longer available. Translations of

the French and Italian texts of Delacroix and Maublanc, and of Ferraris are decidedly obsolete and, of course, were never applicable to new-world conditions to any great extent.

As the title indicates, the work relates primarily to the Antilles; but plant diseases know no political boundaries, so that it is unfortunately true that most of the ills to which the crop plants of the West Indies are heir, likewise occur throughout the tropical and subtropical areas of the New World.

It need hardly be said that the author is particularly well-fitted for the task involved in preparing the book, since he has been actively engaged in the study of plant diseases for many years. Of this time, some 20 years have been spent in Tropical service in Cuba and Puerto Rico of the Greater Antilles. Dr. Cook has been very fortunate in the translator who has collaborated with him, and whose contribution consists of far more than a mere substitution of Spanish words for the English. He has attempted with much success to encompass the spirit of the original and produce a text adequate in all ways to carry out the purpose of the author. In so doing he notes that difficulties were experienced, not the least of which was the lack of technical terms in the language of Castile, which necessitated much extra effort in searching for satisfactory terms.

The author introduces his treatise with a brief history of the subject, outlining it by pertinent references previous to 1900. After a brief account of the physiology of plants, the following 40 pages are devoted to a general discussion of the causes, symptoms, manner of transmission, and the control of plant diseases. The body of the work consists of 442 pages, of which 136, or almost exactly 30 per cent, are devoted to the diseases of sugar cane. This amount of space is not at all out of proportion to the importance of this crop in the Antilles. *Saccharum* and its many diseases have been studied intensively over a long period of years, almost to the exclusion at times of other crops. This condition is reflected in the present work, which summarizes adequately the present state of knowledge of 31 diseases recognized for Cuba and Puerto Rico and gives brief accounts of various other parasitic and nonparasitic diseases reported for other cane-growing countries.

Second in rank are the citrus diseases with 65 pages, followed in order of importance by the banana with 38, cacao with 26, coffee with 15, tobacco with 12, pineapples with 10, the vegetables as a group with 90, minor fruits and other economic plants accounting for the balance. An effort appears to have been made to deal in so far as space limitations permitted not only with the many diseases actually present in the Antilles, but to record those that occur in other parts of Tropical or sub-Tropical America. Some important Oriental diseases, such as the dread coffee rust, also are discussed. Both the author and the translator have been interested in virus diseases, as is evidenced by their published bibliographies on the subject, and the viruses have not been overlooked, particularly in the case of sugar cane, which has most certainly had more than its share of this type of disease.

Diseases due to unfavorable environmental conditions, to nutritional deficiencies, and other parasitic causes are adequately treated. The section devoted to vegetable diseases is of particular interest, since the growing of various of these crops is a comparatively new development, both for home use and export, and the diseases have been much in evidence as efforts have been made to expand vegetable production.

As a pioneer in its field, it is to be expected that errors have crept in, which a later edition can remedy. An unfortunately large number of typographical errors will be found. There are 5 on page 327, for instance. The author has not been consistent in capitalizing specific names in following the International Rules nor in abbreviating authorities for technical names. He has not clearly differentiated between fungi directly parasitic in plants and those attacking insect pests of the plants treated. Similarly *Zygosporium oschioides* is a parasite of *Pucciniopsis* on *Carica* and not directly of *Carica* itself (p. 321). Onion smut (p. 387) is attributed to *Uromyces* rather than to *Ustilago*. Most workers would take issue with the statement that *Stilbella flavida* can be combatted easily "facilmente," with Bordeaux Mixture.

A very disappointing feature is the fact that many of the illustrations have been so poorly reproduced as to be worthless, for which, however, we cannot blame the author.

A bibliography has not been included in the interest of space conservation, and the reader is referred to the author's and translator's recently issued "Bibliography of Mycology and Phytopathology of Central and South America" published in The Journal of Agriculture of the University of Puerto Rico 21: 249-486. 1937. This bibliography is said to be still available from the compilers at the Experiment Station, Rio Piedras, Puerto Rico.

In view of the difficulties encountered by the translator in finding adequate Spanish technical terms and the resulting need for developing a satisfactory Spanish terminology for the sciences, it has been a most commendable plan to include a glossary, which, briefly but adequately, defines each term used in the text and gives an English equivalent.

The book is completely and apparently carefully indexed as to scientific names of parasites and the common names of diseases and hosts. It does not include the scientific

names of the hosts, however, so that the reader, unfamiliar with Spanish common names or with the particular common name used, since these vary greatly from country to country, may experience difficulties in locating a given crop plant.

The work is distinctly timely and will fill a definite need on the part not only of the actual workers in the specialized field, but of those engaged in extension work, an activity now coming into its own in Puerto Rico, and other Latin-American countries, of agriculturists in general, and of all who are in any way concerned with the problems presented by disease in plants.—JOHN A. STEVENSON, Bureau of Plant Industry, Washington, D. C.

HOLMES, FRANCIS O. *Handbook of Phytopathogenic Viruses*. 221 pp. Price \$2.00.  
Burgess Publishing Company, Minneapolis, Minn.

This handbook of plant viruses is a very much enlarged edition of the author's paper on a "Proposal for extension of the binomial system of nomenclature to include viruses" (Phytopath. 29: 431-436, 1939) in which he proposes a latinized binomial system of nomenclature for plant viruses. The present volume includes a classification and nomenclature of only the better-known plant viruses.

The table of contents gives in outline form the author's earlier attempt to classify and name the plant viruses. In the text the viruses that affect higher plants and bacteria are classified under kingdom, divisions, classes, families, genera, species, and varieties. The basis of classification is nearly entirely symptoms, rather than any fundamental characters of the viruses themselves. The family Chlorogenaceae and genus *Chlorogenus* includes the viruses of the yellows diseases, as aster yellows, peach yellows, little peach, potato witches broom, etc., all of which are leaf hopper transmitted; but it does not include all leaf-hopper transmitted viruses. The family Marmoraceae, genus *Marmor*, includes viruses causing mottling or necrotic spotting of the host; and Annulaceae the viruses causing ring patterns, with "recovery" and eventual "non-sterile immunity." The formation of vascular proliferations or galls is the basis for including other viruses in the family Gallaceae. Spindle tuber, leaf curl, leaf roll, dwarf disease, savoy disease, and spotted-wilt families complete the viruses attacking the higher plants.

Each species or variety of the better-recognized viruses is described in detail giving Latin and common names and some synonyms, a partial list of susceptible and sometimes insusceptible species, distribution, induced diseases, transmission, serological, and "immunological" relationships, properties, and control, with a selected list of literature. These descriptions of the viruses should prove valuable for reference purposes. In supplement I, 28 pages are devoted to Bacteriophages of animal and plant bacteria, giving much the same type of information for each species of the genus *Phagus* as is given for the plant viruses. Supplement II lists the hosts for some of the better-known viruses, as aster yellows, curly top, cucumber mosaic, etc., and lists both the hosts and species nonsusceptible to the tobacco mosaic virus. Supplement III lists viruses that for one reason or another are not treated in the handbook. An index follows, in which the page numbers of viruses fully described are underlined. The material throughout appears to be well selected and is put in an orderly, concise form, so that information is readily accessible.

The publication of this book obviously adds to the confusion already present in plant-virus nomenclature, and, one might add, to classification also. Perhaps this is desirable until some system of classification and nomenclature is finally adopted; as one would hesitate when writing about the tobacco-mosaic virus to refer to it as Tobacco virus 1, Johnson; *Nicotiana* virus 1, Smith; *Marmor tabaci* var. *vulgare* Holmes, or common tobacco-mosaic virus; but may be content to refer to it as the tobacco mosaic virus. If the latter course is followed for the present no harm will have been done in placing before those interested in viruses another proposal for naming them, and those interested in classification will have another basis for grouping to consider.

It does not seem that full advantage has been taken of our present knowledge of the viruses in classifying them. For example, the genus *Marmor* includes the tobacco-mosaic virus, the serologically related English cucumber-mosaic virus, the unrelated American cucumber-mosaic virus, the etch virus, and many others that cause mottle symptoms in their respective hosts, and all are coordinate without regard to relationship. It would seem that tobacco-mosaic virus, American cucumber-mosaic virus, etch virus, potato ring-spot virus, and perhaps some other viruses in the genus *Marmor* are distinct kinds and should be given generic rank. Then the English cucumber-mosaic virus and tobacco-mosaic virus could be considered species of a common genus. The vein-banding virus is improperly treated as a strain of the cucumber-mosaic virus. If future studies confirm the claim that it reacts serologically with antigens of cucumber mosaic virus it cannot even then be considered coordinate with a mutant strain of the cucumber virus, but should be considered a distinct species. It should be kept in mind that cucumber-mosaic

virus and vein-banding virus are frequently found associated in the same tobacco plant and that one gives no protection against the other.

The author, possibly without recognizing it, is laying the foundation for endless trouble, if his proposal to name strains of constantly mutating viruses is followed. The laboratory workers may feel justified in naming mutant strains, but to the person working with the field strains the naming of strains of the constantly mutating viruses becomes unthinkable. *Marmor tabaci* var. *vulgare* is the name given to the "typical strain" of the tobacco mosaic virus, with tobacco virus 1 and *Nicotiana* virus 1 as synonyms. By definition these latter terms mean the common field mosaics that have been recognized the past 50 years. Either the author believes that there is only one common field strain, which he names var. *vulgare*, or, if he recognizes the true situation, namely that there are numerous distinct field strains of the common tobacco-mosaic virus, he is willing to classify all of them under one varietal name. The tobacco ringspot and the cucumber-mosaic viruses are also made up of numerous strains in nature and it is questionable whether any one strain of the cucumber-mosaic virus is common enough to be called var. *vulgare*. Recognition of endless variations or mutations of viruses, such as the tobacco-mosaic virus, both in the field and laboratory, is essential, but to name each mutant with other than a laboratory designation will result only in confusion.

Where strains of a virus are well established in nature and cause a well-recognized disease year after year, the use of a varietal name seems justified. For example the little peach virus is named *Chlorogenus persicae* var. *micropersica*. It is questionable, however, whether pathologists will submit to using *Chlorogenus persicae* var. *vulgaris* for the peach yellows virus when *Chlorogenus persicae* will do.

The use of the term "immunity" throughout the text, in referring to failure of a virus to cause a repetition of symptoms in a plant already affected with a slightly different strain of that virus, is unjustified. The plant is not immune from either virus, but is "protected" to a greater or less degree by the first virus against the second.

The reviewer hesitates to express an opinion regarding the advisability of adopting a latinized system of nomenclature for viruses, but it is such a marked advance over any proposal yet offered that it should be given very careful consideration.

Aside from what one may think of the advisability of applying a binomial system of nomenclature to viruses at this time, the book probably will prove a welcome addition to the virus literature, as it gives in a small, compact, loose-leaf type of book a list of viruses of plants and, concisely and apparently accurately, what is known of them.—  
W. D. VALLEAU, University of Kentucky, Lexington, Ky.

## THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

### 1940 SUMMER MEETING

SEATTLE, WASHINGTON—JUNE 19-22

In conjunction with the Program of the American Association for the Advancement of Science and Affiliated Societies, June 17-22.

Wednesday, 9:00 a.m.—Business meeting of Pacific Division of The American Phytopathological Society.

Wednesday, 10:00 a.m.—Presentation of papers.

Wednesday, 1:30 p.m.—Presentation of papers.

Thursday, 9:30 a.m.—Joint session with Northwest Association of Horticulturists, Entomologists, and Plant Pathologists; Economic Entomologists and Society for Horticultural Science.

Invitation papers dealing with field plot arrangement, statistical analysis, virus vectors, virus physiology, or related topics.

Thursday, 1:30 p.m.—Presentation of papers.

Friday, 9:30 a.m.—Symposium on fruit tree virous diseases.

Saturday: Field trip to Western Washington Experiment Station, Puyallup, Washington. Joint program with Horticulturists and Entomologists. Demonstrations of spray equipment and experimental plots.

Pathologists who wish to present papers at these meetings should submit titles to L. D. Leach, Secretary of the Pacific Division of the The American Phytopathological Society, University of California, Davis, California.

CHAS. CHUPP, <i>President</i>	R. S. KIRBY, <i>Secretary</i>	B. F. DANA, <i>President</i>
Cornell University	Pennsylvania State	Pacific Division
Ithaca, N. Y.	College	U. S. D. A.
	State College, Pa.	Corvallis, Oregon

#### SUMMER MEETING OF THE NORTH CENTRAL STATES GROUP OF PHYTOPATHOLOGISTS

The North Central States group of phytopathologists will conduct a summer tour in western Illinois from June 20 to 22. The group will assemble at Quincy, Illinois, on June 19. June 20 will be spent on tree fruit and small fruit diseases near Quincy and on grain diseases in the Illinois River bottom near Jacksonville. The afternoon will be devoted to an inspection of the experimental orchard spraying work at Jerseyville. The night will be spent at the famous Pere Marquette State Park near Grafton.

On June 21, the group will tour the intensive vegetable area in the Mississippi River bottom near East St. Louis where various vegetable and field crop diseases will be seen.

Members of the Society other than those in the North Central States (Michigan, Wisconsin, Minnesota, Iowa, Nebraska, Missouri, Illinois, Indiana and Ohio) who plan to attend the meeting should write Dr. H. W. Anderson for detailed program about May 20.

Committee on Arrangements,

C. M. TUCKER  
I. H. MELHUS  
H. W. ANDERSON

# STUDIES ON THE BIOLOGY OF VALSA SORDIDA AND CYTOSPORA CHRYSOSPERMA<sup>1</sup>

CLYDE M. CHRISTENSEN<sup>2</sup>

(Accepted for publication December 1, 1939)

## INTRODUCTION

The fungi discussed in this paper are among the most common bark-inhabiting fungi found on our native forest poplars and on the introduced species and varieties of ornamental poplars. They have been considered to be of rather minor importance on our native forest trees, but have been thought to be one of the chief reasons for the poor survival and short life of ornamental poplars in this and some other regions. Despite their prevalence and their at least supposed practical importance, comparatively little is known of some phases of their life. The present studies were undertaken to find out certain facts about the growth, reproduction, and parasitism of the fungi concerned and to determine their range of variation.

## SOURCE OF MATERIAL

The sources of the fungi studied are shown in table 1. Unless otherwise stated, the cultures were obtained by suspending masses of spores in sterile water and placing drops of this spore suspension on malt agar slants in test tubes. Special studies were made with single-spore isolates from some collections of the fungi, but, for most of the work, mass spore cultures were used.

## TAXONOMY OF THE GENUS VALSA

The taxonomy of this genus has been reviewed in detail by Schreiner (9), and will be considered here only as it applies to the specific problems in this study. Rabenhorst (7) describes 129 species of *Valsa*, 6 of them on *Populus*, and 11 on willows. Some of these several species he considers as collective. Saccardo (8) lists 133 species of *Cytospora*, 3 of which occur on poplar. He limits *Cytospora chrysosperma* to species of poplar and, more recently, Grove (1) has done the same. Hubert (2) reported this same fungus on 12 species of trees, in 6 different genera, and this host range has been extended by other authors. Neither Grove nor Hubert states his reasons for considering the species as he does, the former listing no characters by which the species could be distinguished with certainty from all other species of *Cytospora*, and the latter making only a categorical statement of the identity of the fungus. Obviously there are at present no very positive means of identifying *C. chrysosperma*. If the limits of variation of this fungus on species of *Populus* were established, one would have a basis on

<sup>1</sup> Paper No. 1751 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

<sup>2</sup> This paper is a summary of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Doctor of Philosophy degree.

TABLE 1.—*Source of isolates of Cytospora chrysosperma and Valsa sordida*

Isolate No.	Host	Location in Minnesota	Remarks
<i>Cytospora chrysosperma</i>			
1	<i>Populus tremuloides</i>	University Farm	Living tree.
2	do do	Afton	Isolated from wood near an insect tunnel.
3	do do	Itasca Park	Isolated from wood near Monochamus tunnel in interior of log cut the previous year.
4	<i>P. candicans</i>	University Farm	Dead twigs of living tree.
5	<i>P. alba</i> , var.		do
	<i>pyramidalis</i>	Lake City	do
7	do do	Minneapolis	do
8	<i>P. spp.</i> (Russian poplar)	Cloquet	Canker on living tree.
9	<i>Juglans</i> spp. (Japanese walnut)	University Farm	Dead twig of living tree.
12	<i>P. alba</i>	Minneapolis	do
14	<i>P. alba</i> var. <i>nivosa</i>	St. Paul	Canker on living tree.
17	<i>P. spp.</i>	Savage	Dead branch of living tree.
19	<i>P. spp.</i> (exotic hybrid)	Excelsior	do
20	<i>P. nigra</i>	Minneapolis	do
23	<i>Ulmus americana</i>	Unknown	Tissue culture from dead branch.
26	<i>P. nigra</i>	Brainerd	Dead branch of living tree.
28	do	St. Paul	do
<i>Valsa sordida</i>			
32	<i>Populus tremuloides</i>	Anoka	Dead tree.
33	do	Wyoming	Dead branch of living tree.
37	do	Unknown	do
39	do	Anoka	do
40	do	do	do
41	do	do	do
42	do	do	do
43	do	do	do
44	do	do	do
46	do	do	do

which collections of the fungus from other hosts could be compared. To establish such a basis it has been necessary to study in considerable detail a number of different characters of the fungi concerned, to find out which of those characters are sufficiently constant and characteristic to be of diagnostic value, and to determine their range of variability. It should thus be possible eventually to make some order out of the taxonomic chaos in which these fungi are involved. The elucidation of the relationship between *C. chrysosperma* and *V. sordida* is a necessary part of this work.

#### Valsa sordida

**Development and Structure of Pycnidia** The pycnidium begins as a clump of densely interwoven hyphae that at first is covered by a layer of bark from 6 to 20 cells deep. At about the time the tip of the pycnidium emerges from the bark, a cavity begins to form in the lower central part of the interior of the stroma. Usually, if indeed not invariably, only one

cavity is initiated. This continues to enlarge in a very irregular manner, so that eventually a cavity is formed that contains many interconnected chambers of various shapes and sizes. There is a wide variation in the shape of these chambers, and 2 pyrenidia, growing side by side on a piece of bark inoculated with a pure culture, may differ considerably. The outer wall comprises several layers of pseudoparenchymatous cells. Viewed from the outside the mature pyrenidium is irregular in shape, the convolutions of the wall conforming roughly to the shape of the larger chambers within.

Conidiophores arise from the inner layer of cells that form the wall of the chamber. These are filiform, colorless, usually unbranched, but occasionally branched once, and sometimes twice. They are from 10 to 18  $\mu$  long and about 1  $\mu$  in diameter. Spores are produced at the ends of the conidiophores. A spore first appears as a terminal swelling; this grows until the typical spore length is attained. The conidiophore becomes constricted at the base of the spore, and this constriction increases until the spore is cut off. Although the continued production of conidia by a single conidiophore has not been observed, it seems obvious, from the quantity of spores produced, that each sporophore continues to function for some time.

*Development of Pyrenidia on Sterilized Twigs.* Most isolates, growing on sterilized twigs in flasks, form fewer but larger pyrenidia than when growing on the bark of trees in the field. If the bark is not too moist the pyrenidia develop normally within the bark; if excess water be present they may form on the surface of the bark, much as they do on the surface of an agar medium. Spores are exuded from some of these pyrenidia in coiled yellow spore horns. In these flasks, even when kept in a laboratory where the temperature fluctuates 5° C. or more, there can be comparatively little change in relative humidity. Thus it seems likely that in this case the spores are exuded not so much as a result of swelling of the gelatinous matrix, but rather because the spores and matrix are produced continuously and in such quantity that they are forced out. When the tips of mature pyrenidia are cut off a tendril of spores oozes out at once, and the speed with which it appears indicates that there is some pressure within the pyrenidium. The prolificacy of the fungus is amazing. On a small twig having a surface area of approximately 30 sq. cm. more than 25 billion spores were exuded in about a week, when the cultures were between 20 and 28 days old, and probably half that number remained within the pyrenidia.

Freehand sections were cut transversely through a number of pyrenidia of each ascospore isolate. No constant morphological differences were found between the pyrenidia of the different isolates, and the variation between different pyrenidia of one isolate seemed just as great as between pyrenidia of different isolates. A diagram of a typical one is shown in figures 1, B and C.

*Size of Conidia.* Fifty conidia of each of 5 ascospore isolates of *Valsa*

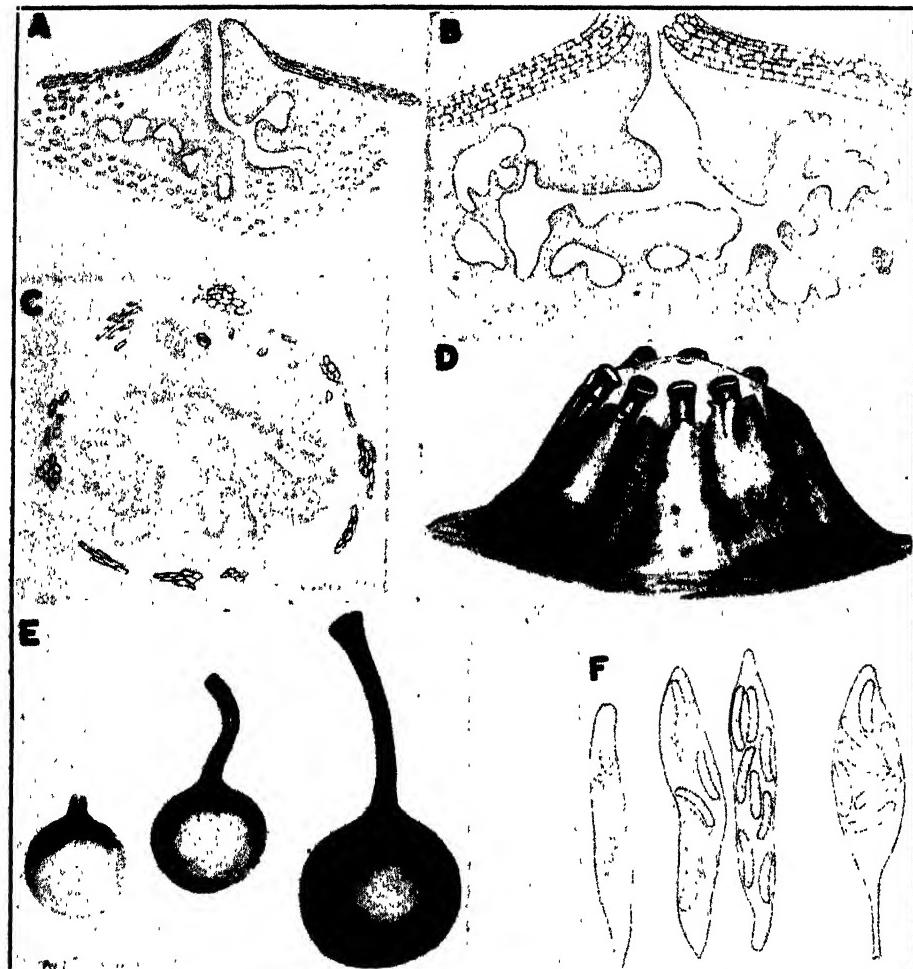


FIG. 1. A. Vertical section through the center of pyenidium of *Cytospora chrysosperma*. B and C. Vertical and cross sections, respectively, through pyenidia of *Valsa sordida*. D. A typical group of perithecia of *Valsa sordida*, the necks coming up around the old pyenidium. E. Perithecia in different stages of development. F. Ascospores, immature on the left, mature on the right.

*sordida* were measured. The spores were taken from pyenidia produced on malt-agar slants in test tubes, kept in the laboratory. To avoid the possibility, however remote, of getting immature spores, only those spores were taken that had been exuded from the pyenidia. They were suspended in water, a drop of the suspension was placed on a slide, a small drop of warm agar added, and a cover glass applied quickly and pressed down firmly. This method was used because the apparent size of spores was altered significantly by mounting them in some other solid media, in lactophenol, or even staining them with cotton blue. It was found necessary to use some comparatively solid medium, to prevent flowing and Brownian movement, if large numbers of spores were to be measured

accurately. The diluted agar mount did not alter perceptibly the apparent length of the spores. They were measured with a screw micrometer, using an oil immersion lens (Table 2). There is detectable very little difference in length of spores from the different isolates.

TABLE 2.—*Length of conidia of different isolates of Valsa sordida on Populus tremuloides and Cytospora chrysosperma on other species of Populus*

Isolate	Host	Average length of 50 spores, in microns
<i>Valsa sordida</i>		
40	<i>Populus tremuloides</i>	4.49
41	Do	4.52
42	Do	4.62
44	Do	4.49
46	Do	4.62
<i>Cytospora chrysosperma</i>		
4	<i>P. candicans</i>	5.73
7	<i>P. alba</i> , var. <i>pyramidalis</i>	5.64
17	<i>P. sp.</i> (probably <i>P. alba</i> )	4.44
19	<i>P. sp.</i> (exotic hybrid)	3.90
26	<i>P. nigra</i> , var. <i>italica</i>	4.06

*Structure and Development of Perithecia.* Pyrenidia, borne on trees in the field, cease to function after a relatively short but very productive life, and the walls disintegrate slowly. Perithecia arise below and in a circle around the old pyrenidia. From 1 to 20 perithecia may arise around a pyrenidium, the typical number in the material studied being 6 to 8. They arise among the bark cells, and are not surrounded by any definite stromatic tissue, merely sitting loosely in the disintegrated bark. A sketch of the perithecia is shown in figure 1, D and E. The enlargement at the top of the neck of the mature perithecium is typical; frequently adjacent perithecia in a group are grown together at this enlarged portion and may be lifted out of the bark together.

Asci arise as outgrowths from pseudoparenchymatous cells composing the inner wall of the perithecium. All the spores in an ascus are approximately the same size, and the variation between spores in different asci is far greater than that between spores within an ascus. There are typically 8 spores in an ascus, occasionally 7, and rarely 6. It has not been noticed that any of the spores, when fewer than 8 are present, are abnormally large. They usually are arranged in a biseriate fashion in the ascus, but this varies a good deal, and often spores are irregularly distributed in the ascus. The spores appear larger when they are first delimited than when they are mature, and the mature ones are quite clear.

The ascus wall is surrounded at all times during its growth by a rather thick gelatinous sheath, which becomes invisible, if present, in many mature asci. Typical asci are shown in figure 1, F. When the ascus is mature it is liberated from the wall of the perithecium. The spores may be lib-

erated in any one of three different ways: 1. Some ascospores break within the peritheciun, and the spores escape, much as do the conidia from the pycnidium, by oozing out, except that the matrix in the perithecia is less gelatinous and less abundant than that produced by the pycnidia. If a mature or over-mature peritheciun be broken or cut open, a large number of free ascospores are found within, although very old perithecia may be completely empty. The spores exuded from a fresh peritheciun collect around the ostiole in sticky, white masses. 2. Some ascospores are forced up the neck to the ostiole, where they burst, discharging the spores forcibly into the air. Pieces of bark, collected in March, and bearing mature perithecia of *Valsa sordida*, were placed on moist cotton in a Petri dish, arranged so that the ostioles of the perithecia were about 5 mm. from the cover of the dish. After about 24 hours a deposit of spores, visible to the naked eye, appeared on the glass above one group of perithecia. The spores discharged from each peritheciun were in a separate clump. 3. Many of the ascospores that travel up the neck of the peritheciun do not project the spores into the air when they burst, and these collect around the ostiole of the peritheciun. In the material observed many more spores collected around the ostioles than were shot out into the air, but the manner of liberation must depend considerably upon the amount of water present.

The perithecial stage has been found by the writer only on *Populus tremuloides*. Perithecia were found on 16 of 30 specimens of *P. tremuloides* collected near Anoka, Minnesota. Perithecia also were found in abundance on specimens collected at the University Farm, Carlton, Wyoming, and Sandstone, which indicates that it probably is formed commonly through central and northern Minnesota, and is not so rare as has previously been stated.

*Size of Ascospores.* One hundred ascospores from perithecia on each of 5 different specimens collected in an area less than 100 yds. square, near Anoka, were measured with the aid of a screw micrometer. The spores from each specimen were taken from the perithecia around one pycnidium. The perithecia were crushed in a drop of water to obtain a suspension of spores, a small quantity of this spore suspension was placed on a slide, some warm liquid agar added, and a cover glass applied and quickly pressed down. The average length of 100 spores from each of the 5 specimens was 8.2, 8.3, 8.9, 9.9, and 10.4 microns, respectively. The minimum significant difference between any two averages was 0.7  $\mu$ . The spores from each specimen were remarkably constant in length, as determined by comparing the average length of the first 50 measured with the average length of the second 50 measured. For example, in collection 45 there was only 0.5  $\mu$  difference, and in collection 34, 0.1  $\mu$  difference between these two averages. Using twice the standard error of the difference as a criterion of the minimum level of significance, there was a significant difference in length between the spores from some of the different collections. Naturally, the cause of this difference is not known, and it cannot be assumed arbi-

trarily that the differences are inherent and that these collections constitute different races. Judging from the above results and from the figures given by other workers, the ascospores of *Valsa sordida* vary considerably in size. If spore length is to be used as one of the criteria of species of *Valsa*, as it has been, this variability obviously must be taken into account.

*Cultural Characters.* These fungi were cultured chiefly to find out if *Valsa sordida*, from *Populus tremuloides*, could be distinguished from *Cytospora chrysosperma* from other species of *Populus*. The isolates were grown first in test tubes, then repeatedly transferred to detect the presence of other organisms. The first transfer usually was made by taking freshly exuded spores from a pycnidium, suspending the spores in sterile water, and placing a drop of this spore suspension on a malt-agar slant. In most cases this technic insured cultural purity. So far as the writer was able to see, there was no consistent difference in cultural characters between the isolates of *V. sordida*, from aspen, and the isolates of *C. chrysosperma* from other species of poplar. Photographs of cultures of several isolates of *V. sordida* are shown in figure 2, A.

#### RELATIONSHIP OF CYTOSPORA CHRYSSPERMA AND VALSA SORDIDA

Numerous authors have followed Nitschke (5) in stating that *Cytospora chrysosperma* is the imperfect stage of *Valsa sordida*. The writer has not found any statement that *V. sordida* has been obtained from *C. chrysosperma*, which should be done to have positive proof of the identity of the fungi concerned. Naturally, it is of some importance to know whether the *Cytosporas* commonly found on ornamental poplars actually are identical with the *Valsa* and *Cytospora* on aspen, or whether two or more fungi are concerned.

Seymour (10), in his host index of the fungi of North America, lists *Valsa sordida* on *Populus tremuloides* and *P. angustifolia*. In the mycological herbarium in the Department of Plant Pathology and Botany, University of Minnesota, the writer examined the following specimens of *V. sordida*: 1. On *Populus* spp. from Decorah, Iowa, collected by E. W. Holway; 2. On *P. pyramidalis*, from Treplitz, Bohemia, Austria, collected by de Thümen. There are 3 pieces of bark in this collection. One bears pycnidia of *V. nivea*, another bears pycnidia, apparently of *V. sordida*, and there are no visible fruiting bodies of any fungus on the third; 3. On *P. nigra*, from Königstein, Germany, collected by W. Krieger. There are 2 specimens, 1 bearing pycnidia, apparently of *V. sordida*, and the other pycnidia and perithecia of *V. sordida*; 4. On *P. tremulae*, from Russia, collected by Ligit Serebriannikov. Only one specimen is present and this bears perithecia of what appears to be *V. nivea*. 5. On *P. nigra*, from France, collected by F. Fautrey. This specimen bears perithecia of *V. sordida*. The writer has found the perfect stage only on *P. tremuloides*.

There are several possibilities to account for the fact that perithecia of *Valsa sordida* are found so frequently on *Populus tremuloides*, and infre-

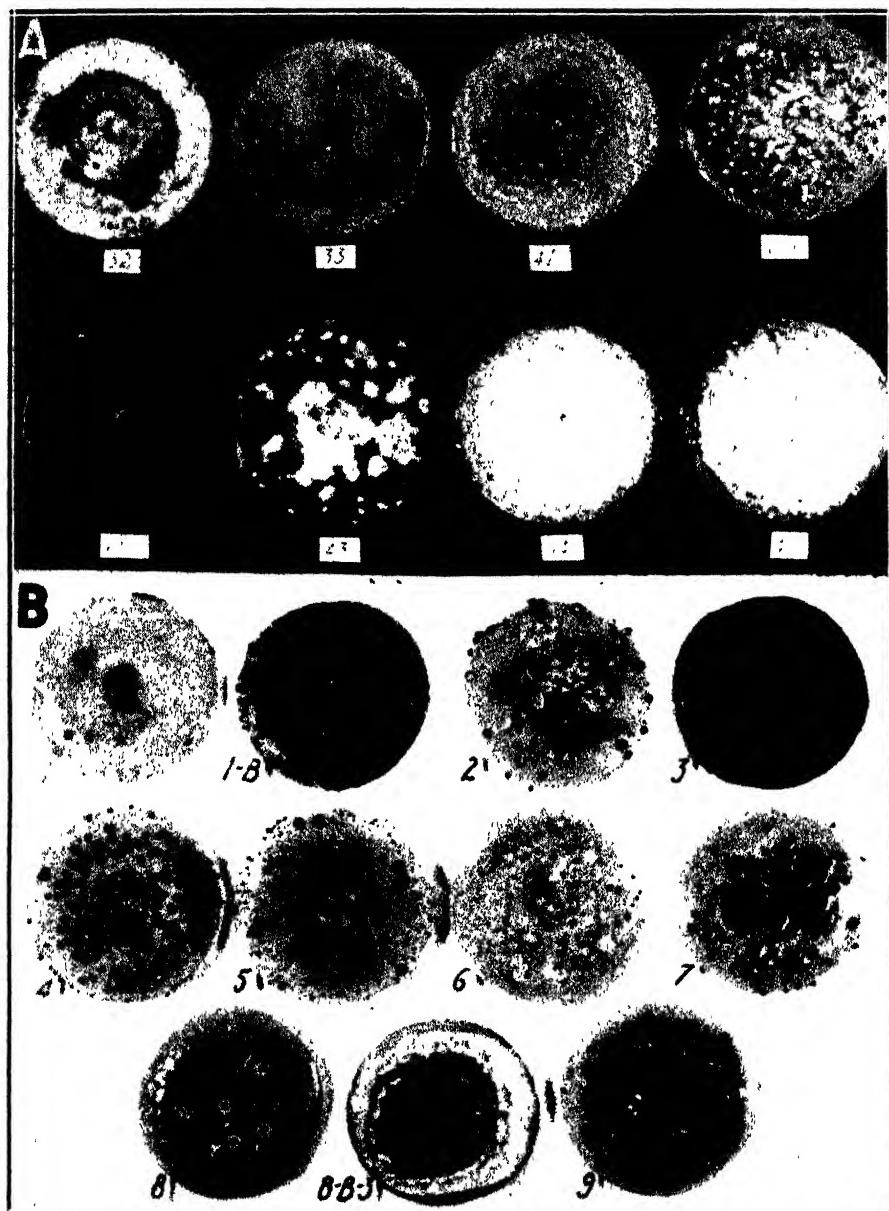


FIG. 2. A. Isolates of *Valsa sordida*, from *Populus tremuloides*. B. Isolates of *Cytospora chrysosperma*: 1, *Populus tremuloides*, Univ. Farm; 1-B, a variant of 1; 2, *P. tremuloides*, Afton; 3, *P. tremuloides*, Itasca Park; 4, *P. candicans*, Univ. Farm; 5, *P. alba* var. *pyramidalis*, Lake City; 6, *Salix discolor*, Itasca Park; 7, *P. alba*, Minneapolis; 8, Russian poplar, Cloquet; 8-B-3, a variant of 8; and 9, Japanese walnut, Univ. Farm.

quently on the bark of other species. 1. *Cytospora chrysosperma* and *V. sordida* are identical, but perithecia are produced only under certain environmental conditions that rarely are encountered on some species and varieties of poplar. 2. There are different strains of the fungus, some of

which are more common on aspen, and readily produce perithecia, while others are more common on other species of poplars and rarely or never produce perithecia. 3. The fungi are taxonomically distinct, although the pycnidia are similar. The writer attempted to settle this problem by trying to induce the isolates of *V. sordida* and *C. chrysosperma* to form perithecia, which would be the only positive proof, by studying the development and morphology of the pycnidia and asexual spores, and by comparing the cultural characters.

*Attempts to Induce the Formation of Perithecia.* Several isolates of *Cytospora chrysosperma* and *Valsa sordida* were inoculated onto sterile aspen bark, and the resulting cultures were subjected to alternate freezing and thawing and were alternately dried and moistened. Some were kept for over 2 years. A few were exposed to ultraviolet light. Other organisms that commonly grow in old aspen bark were added to some of these cultures. No perithecia were formed on any of the bark cultures. Several isolates were grown on the medium described by Leonian (3) as favorable to the production of perithecia of *V. leucostoma*, but none of the writer's isolates formed perithecia on this medium.

#### COMPARISON OF THE STRUCTURE OF PYCNIDIA OF CYTOSPORA CHRYSSOPERMA AND VALSA SORDIDA

Pieces of bark from several different collections of *C. chrysosperma* from species of *Populus* other than *P. tremuloides* were embedded in paraffin, sectioned with the microtome, and examined microscopically. A diagram of a section through the center of one is shown in figure 1, A. No consistent difference could be found between these pycnidia and those of *V. sordida* on aspen.

Seven isolates of *C. chrysosperma*, from several species of *Populus*, were grown on sterile bark in Erlenmeyer flasks, and freehand sections of numerous pycnidia of each isolate were compared with those cut from pycnidia of *V. sordida* grown in the same way. No consistent difference was observed.

*Length of Conidia of Cytospora chrysosperma.* Fifty spores of each of 5 isolates of *C. chrysosperma* were measured, using the same technic as that previously described in measuring the spores of *Valsa sordida*. The results are given in table 2. The range in size is greater than was found for *V. sordida*, but the conidia of the latter fungus cannot be separated from those of *C. chrysosperma* as a group on the basis of size.

*Growth of Cytospora chrysosperma on Sterile Twigs.* Several isolates of *C. chrysosperma*, inoculated into the bark of logs kept in a large moist chamber, produced a very abundant crop of pycnidia within 2 weeks. These were slightly larger than those produced on trees in the field, and some of them exuded long tendrils of spores. The spores in the tendril illustrated in figure 3, C were suspended in water and sufficient samples of the suspension counted in a Spencer counting chamber to permit at least a reasonably accurate estimate of the number of spores present. This one tendril contained about 580,000,000 conidia.

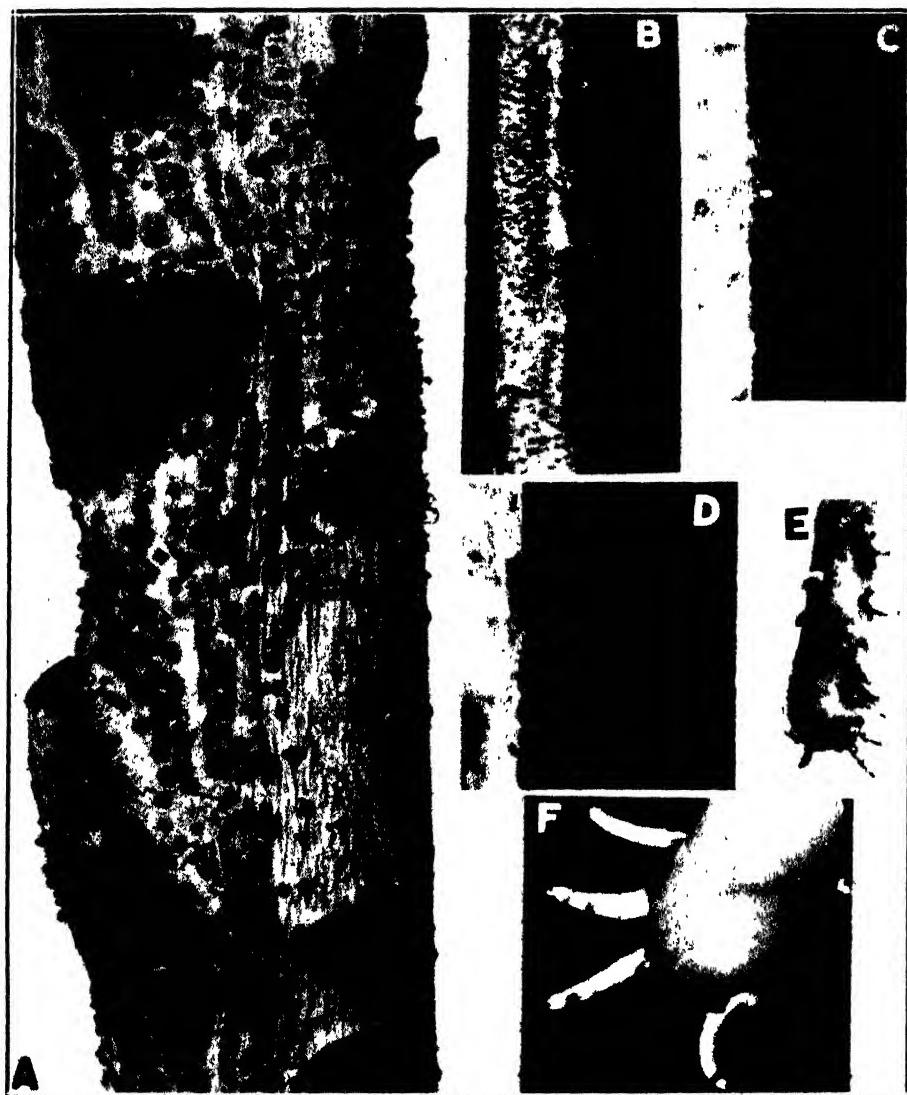


FIG. 3. A. *Cytospora chrysosperma* on poplar. B. On willow. Explained in text. C and D. Spore tendrils of *Cytospora chrysosperma* growing on aspen bark in a moist chamber. E and F. Pyrenidia of *C. chrysosperma* on malt-agar slants in test tubes.

**Growth on Agar Media.** Cultures of most of the isolates produce pyrenidia on malt agar, and the different isolates can be distinguished from each other readily by the size, shape, color, abundance, and time of production of pyrenidia. While there is no one cultural character by which the species can be recognized, the production of pyrenidia is typical of most isolates, although even individual variants of one isolate may differ greatly in this respect. Other species of *Valsa* and *Cytospora* also produce pyrenidia of the same general character in culture, so that this alone can not be used as an identifying character.

Cultures of a few isolates, and particularly one of the variants of Isolate 1, produced peculiar, elongate pycnidia, several of which are illustrated in figure 3, E and F. Apparently, this development is due to the fact that the pycnidia arise in such a position that the tip presses against the wall of the test tube. The elongation of the pycnidium is equal to the shrinkage of the agar, and the continued pressure of the glass against the pycnidium stimulates growth in length. This was further illustrated by dropping a cover glass on top of 3 newly formed pycnidia on a culture in an Erlenmeyer flask. The 3 pycnidia under the cover glass continued to grow in length for several weeks after the other pycnidia in the culture had ceased to elongate visibly. This growth response to pressure obviously is a rather useful character from the standpoint of the fungus, since it is thus that the pycnidium is stimulated to break through the bark when growing on its natural hosts. Even when young pycnidia are covered by more than 20 layers of bark cells they are bound to break through, as they begin to elongate in the direction in which the bark gives way, continue to grow so long as pressure is exerted upon them, and cease growing soon after the pressure has been released.

All isolates were grown repeatedly on malt agar in Erlenmeyer flasks. The cultures of some isolates of *Cytospora chrysosperma* could not be distinguished from the cultures of some isolates of *Valsa sordida*. The variation between the different isolates of *C. chrysosperma* was greater than that between the isolates of *C. chrysosperma* as a group and those of *V. sordida* as a group, so that the two groups could not be separated by the cultural characters observed in this study. Similarity of cultural characters does not necessarily imply taxonomic identity, but it certainly does suggest that there is no very profound difference between *V. sordida* from *Populus tremuloides* and *C. chrysosperma* from other species of poplar.

*Cultural Variation.* Twenty single spores of Isolate 5 were isolated, and the cultures that developed from these were compared with each other and with the parent culture. When grown on agar in test tubes and flasks the different single-spore strains were remarkable chiefly for their uniformity of appearance; not more than two of them could be distinguished from the others or from the parent culture with any degree of positiveness.

It was observed that Isolate 8 in culture produced numerous pycnidia that differed from each other in size and shape. Mass spores were taken from several different pycnidia and grown separately, and the resulting cultures were found to be easily distinguishable from each other on the basis of the general appearance of the mycelium, time of formation of the pycnidia, and number and size of the pycnidia. Since it is not known how the pycnidia arise, and since the possibility exists that the original isolate may have been a mixture of strains, it is not known whether these obviously different strains were obtained merely by selecting previously existing strains from a mixed culture or whether they arose as variants when the parent culture was grown in the laboratory. No attempt was made to

pursue this phase of the problem further; it is mentioned only to illustrate the kind and extent of variation that may appear in cultures of this organism. Cultures of *Cytospora chrysosperma* from species of *Populus* and from some other host genera, and some of the variants obtained from these cultures are shown in figure 2, B.

#### PATHOGENICITY

Statements by Grove (1), Long (4), Hubert (2), Schreiner (9), and Povah (6) indicate that *Cytospora chrysosperma* can parasitize only more or less weakened trees. In Minnesota it undoubtedly is one of the most prevalent fungi fruiting on the bark of recently dead aspen in the forest, but only seldom has the writer found it apparently causing definite cankers on seemingly healthy, vigorous aspen growing in the forest. It seems doubtful if it is or will be of any considerable importance in naturally reproduced forests in this region. However, several nurserymen in Minnesota have expressed the opinion that the canker caused by this fungus is one of the greatest factors with which they have to contend in growing ornamental poplars. In 1936 one nursery reported 75 per cent of its stock of ornamental poplars killed by this fungus, and every one of the several nurseries visited by the writer in 1935 and 1936 suffered considerable loss of poplars, which the growers attributed to *Cytospora* canker. It is by far the most common fungus found on the dying and dead branches of ornamental poplars planted in this region, and frequently it forms definite cankers on the limbs of these trees. Such cankers have been assumed to be proof of the pathogenicity of the fungus, but they remain only circumstantial evidence.

#### Experimental Evidence of Pathogenicity

Hubert (2) inoculated 5 groups of poplar trees and cuttings with the fungus. One group contained 2 trees, another 3 trees, and the remaining groups 3 cuttings each. All inoculated plants except 2 trees died in from 3 to 6 months after having been inoculated, and pyrenidia of *Cytospora* were formed on the dead bark. No checks were mentioned, and this, in addition to the small number of trees used, made the results of limited value. The fact that pyrenidia of *Cytospora* were found on cuttings that previously had been inoculated with the fungus, does not prove that the fungus used was parasitic, or even that any parasitism was involved. The writer has found that pyrenidia of *Cytospora* will develop, without inoculation, on cuttings of apparently healthy trees when the cuttings are placed in the laboratory or greenhouse and left for a few weeks. This will be referred to later.

Schreiner (9) inoculated 10 to 15 one- and two-year-old trees, growing in the field, each week from the first week in March until the end of May. In that same summer 50 per cent of the trees under  $\frac{3}{4}$  in. diameter (presumably the weaker ones) and 5 per cent of the larger, more vigorous trees that he had inoculated were killed, supposedly by the fungus. Others died in succeeding years. Cuttings of several poplars were inoculated in the

greenhouse, and the dormant ones were found more severely injured than those that were growing.

On June 8 and 9, 1936, the writer inoculated 40 aspen saplings, each from 3 to 5 inches in diameter with 30 different isolates and single-spore cultures of *Cytospora chrysosperma*. Inoculations were made by macerating an area of bark about  $\frac{1}{2}$  cm. square, placing a water suspension of spores and mycelium from an agar slant on this wound, covering this with a bit of moist cotton and wrapping it with paper tape. Only a very few small cankers were formed, and, even after 2 years, none of the inoculated trees had died. None of the check wounds became infected, perhaps because of the method of bandaging the wounds so that conditions favorable to the formation of callus tissue by the bark were maintained. On the other hand, typical *Cytospora* cankers developed around some of the wounds made in the bark when the trees were labelled by scratching numbers into the bark with a small chisel-like instrument, and these cankers doubtless originated from mycelium already present in the outer bark of the trees.

#### Natural Occurrence of *Cytospora* in the Bark of Healthy Poplars

During the course of the investigations a number of sections of several aspen trees were cut, brought into the laboratory, washed with hot tap water and soap, sponged with alcohol, then covered with a thin coat of hot paraffin. This treatment should have removed or killed any *Cytospora* spores on the surface of the bark. The logs were put in a fairly humid chamber, and, within 2 weeks, pycnidia of *C. chrysosperma* appeared over the greater part of the bark in such numbers that much of the paraffin layer was pushed off. Several cuttings from *Populus alba*, from trees that to all outward appearance were healthy, were placed in water in the laboratory, under much the same conditions, apparently, as the cuttings of *P. trichocarpa*, inoculated by Hubert. Two of these did not sprout, and both soon bore a very abundant crop of pycnidia of *C. chrysosperma*. The fungus doubtless was generally present in the bark of at least one of these, since pycnidia appeared everywhere on the surface at about the same time. A typical "canker" was formed on the other, if a canker can be said to form on a dead twig. Pycnidia of the fungus developed also on the dried tops of those cuttings that grew, and formed "cankers" there, but did not grow down from the dead tip, above the uppermost sprout, into the living tissue. Obviously, the fungus was not parasitic in this case. Eight branches of willow with no signs of *Cytospora* on the bark were brought into the laboratory in January, washed with soap and warm water, sponged with 70 per cent alcohol, covered with warm paraffin, and placed under a bell jar with the lower ends in a glass of water. *Cytospora* fruited abundantly on all of them. No inoculation was necessary. The fungus already was present, and became evident only after the twigs died. A fairly typical one of these was photographed (Fig. 3, B). A section of a living branch from a mountain ash (*Sorbus aucuparia*) was brought into the laboratory at the same time and placed

under a bell jar, with the lower end of the section in water. After almost a month had elapsed, no pycnidia of *Cytospora* had appeared on this branch; the bark, when cut into, appeared green and still living. The branch then was placed outside for several hours, until the bark was frozen sufficiently to kill it, and again placed under the bell jar. Within a week typical sunken, discolored cankers appeared on the bark of this branch, and pycnidia of *Cytospora* appeared throughout these "cankers." In this case the fungus, although present, was unable to produce any visible effects even in bark that must have been on the verge of death, and only after the bark was quite dead could the fungus produce cankers and fruit bodies.

A group of *Populus alba* trees was planted on the campus in the spring of 1938, located where the writer passed them almost every day and thus had a good opportunity to observe them closely. Three of these trees died within about 4 months after being planted. Although there was no sign of *Cytospora* canker on the trees when the first inconspicuous symptoms of impending death became evident, pycnidia appeared generally throughout the bark of trunk and branches of one of them soon after the leaves became dry. This tree was cut and the base of the trunk placed in water, with the result shown in figure 3, A. One who examined the tree for the first time after *Cytospora* had appeared could have supposed the fungus to have killed it. *Cytospora* pycnidia appeared on some of the limbs of the other two dead trees. Several limbs of these two trees, none of them with any evidence of pycnidia, were placed with one end in water and within two weeks a most abundant crop of pycnidia was produced on the bark. When in need of good specimens of *Cytospora* to show to his students, the writer merely cuts branches of ornamental poplars or of forest willows, places them in water, and always obtains excellent specimens of the fungus. No inoculation is necessary. The fungus appears to be generally present in the healthy bark of poplars, willows, and some other species of trees in this region.

The foregoing observations and experiments have made the writer somewhat dubious of the actual parasitism involved in many cases of canker formation by *C. chrysosperma*. Sunken areas formed in the bark where the fungus is growing vigorously may be due chiefly to the fact that the fungus has digested portions of the tissues—it does not mean that the nonsunken portion surrounding the canker necessarily is healthy. Thus, when twigs are placed in water, the fungus forms a canker if it is only of local extent in the bark, otherwise it may fruit all over the surface of the twig without forming any definite canker.

There is no doubt that most of the clones of ornamental poplars introduced from Europe and grown in Minnesota are poorly adapted to the soil and climate here. Observational and even experimental evidence indicates that *Cytospora chrysosperma* attacks weakened trees, but if it is generally present in the bark of trees so ill adapted to their environment as many aspen and native willows on poor sites, and many introduced varieties and

clones of ornamental poplars and mountain ash in this region, it must be a weak parasite indeed to permit such trees to survive at all. The writer certainly considers it an open question whether many of the trees seemingly parasitized by *C. chrysosperma* would survive were the fungus not present. The fact that many ornamental poplars succumb the first or second year after they have been transplanted would also tend to emphasize the question as to whether, at least in this region, they die because they are invaded by *C. chrysosperma* or whether *C. chrysosperma*, normally present in the bark, fruits on them because they are dying. The writer does not wish to imply that he believes *C. chrysosperma* unable ever to grow as a parasite, but certainly at times it has been incriminated on insufficient evidence. The writer is of the opinion that the poor survival of ornamental poplars in this region is due less to *Cytospora* than to the fact that these poplars are exotic trees growing in soils and subjected to climatic conditions to which they simply are not adapted. The practical conclusion to be drawn from this is that, so far as the control of *Cytospora* canker on ornamental poplars is concerned, it would be far more to the point to try to develop varieties better adapted to the locality in which they are to be grown, rather than to seek specific prophylactics or remedies for this disease alone.

#### DISCUSSION

It was not possible to find any consistent difference in shape, size, or structure of the pyrenidia, or shape, size, or manner of production of conidia between *Valsa sordida*, from aspen, and *Cytospora chrysosperma* from other species of poplar. At no time could the cultures of the different isolates of *C. chrysosperma* as a group be distinguished from the cultures of isolates of *V. sordida* as a group. There were constant and characteristic differences between different isolates, as perhaps would be expected, but the cultures of some isolates of *C. chrysosperma* were almost identical with those of some isolates of *V. sordida*. From these results it seems fairly safe to state that those strains of *C. chrysosperma* growing on species of *Populus* other than *P. tremuloides* fall within the range of variability, in the characters studied, of the imperfect stage of *V. sordida*. The writer has examined, but has not studied thoroughly, the morphologic and cultural characters of some isolates of *Cytospora* from elm, walnut, willow, and a few other hosts and, at present, he has found no good basis for separating these from *C. chrysosperma* on *Populus*.

*Cytospora chrysosperma* varies considerably in most of the characters studied. The length of conidia seems to vary within relatively narrow limits, but the conidia of what are considered to be other species of *Cytospora* fall within these limits, so that this character alone is not a distinguishing character—or some of the species are not valid. The shape and microscopic structure of the pyrenidia, though rather variable, are fairly characteristic, but *Cytosporas* considered to be other species have essentially the same structure. The manner in which the pyrenidial stroma of *C.*

*chrysosperma* is borne in the bark of the host is typical of a large number of what have been considered other species. The spore tendrils are supposed to be rather large and yellow, but this depends greatly on environmental conditions. Fresh tendrils usually are yellow, but the writer has found *C. chrysosperma* on *Populus alba* and *P. nigra* with red spore tendrils, and when these were grown in culture the tendrils were yellow. Spores of both *C. chrysosperma* and *V. nivea* are exuded from old pycnidia in white masses, so evidently the color fades. The essence of the foregoing is that the writer has not yet been able to find any good diagnostic character of the species.

The fact that the fungus is a more or less normal, though unseen, inhabitant of the bark of apparently healthy poplar, willow, and mountain ash trees, and does not fruit until the trees are dying, makes it difficult to judge how much of a factor it may be in the death of these trees. The writer believes he has a fairly good basis for suggesting that the fungus often is not responsible for the injury with which it is associated.

#### SUMMARY

The pycnidia and conidia of *Valsa sordida* developed in the field and in the laboratory could not be distinguished from those of *Cytospora chrysosperma* produced under comparable conditions.

Isolates of *V. sordida* and *C. chrysosperma* differed among themselves in culture, but there was no consistent difference between those of the former as a group and those of the latter as a group.

Collections of *Cytospora* from hosts other than *Populus* could not be distinguished from collections of *C. chrysosperma* from species of *Populus* when compared as to structure of pycnidia, rate of growth on agar, and general cultural characters.

*C. chrysosperma* is a common inhabitant of the bark of apparently healthy *Populus* trees, especially *P. tremuloides* and *P. alba*, and probably also of willow and mountain ash. The degree of its parasitism on these trees is considered open to question.

It is suggested that the problem of control of *Cytospora* canker on ornamental poplars should be approached by attempting to develop varieties of trees more suited to their general environment than the present ones.

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## THE CHEMISTRY OF RESISTANCE OF PLANTS TO PHYMATOTRICHUM ROOT ROT. V. INFLUENCE OF ALKALOIDS<sup>1</sup> ON GROWTH OF FUNGI<sup>2</sup>

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### INTRODUCTION

The presence of alkaloids in plants has been suggested (5) as an important factor in the mechanism of their resistance to *Phymatotrichum omnivorum* (Shear) Duggar. This was done by correlating the presence or absence of alkaloids in plants with resistance of the species to the fungus as reported by Taubenhaus and Ezekiel (16). This view was strengthened by demonstrating that *Mahonia trifoliolata* Fedde, *M. swaseyi* Fedde and *Sanguinaria canadensis* L. contained large quantities of alkaloids toxic to the fungus and so located in the plant as to account fully for the resistance of the plant (6, 7). To further test the hypothesis, 62 different alkaloids from plants differing in their susceptibility to *P. omnivorum* have been studied by incorporating them, in various concentrations, into the substrates of the medium ordinarily used for pure culture of the organism. In addition, for comparative purposes, the influence of 6 alkaloids on 6 fungi other than *P. omnivorum* has been determined.

### LITERATURE REVIEW

The influence of alkaloids on the growth of fungi has been the subject of several investigations. Bates (1) studied the effect of strychnine, quinidine, and caffeine on the growth of *Aspergillus niger* and *Rhizopus nigricans*. He used concentrations of the alkaloids up to 1.8 per cent. Bates found that strychnine sulphate increased growth of *R. nigricans* and of *A. niger* in Coons nutrient solution, and quinidine sulphate had a similar effect on the latter organism. Growth of both fungi was decreased by caffeine citrate and that of *R. nigricans* by quinidine sulphate. These 3 compounds also retarded sporulation of *A. niger*.

Marcacci (9) found that lactic acid fermentation was accelerated by the presence of atropine and morphine but was retarded by quinine, veratrine,

<sup>1</sup> For the sake of convenience, the term "alkaloid" is used in its broader sense to include the purines and some of the simpler naturally occurring nitrogen bases.

<sup>2</sup> Approved by the Director as Contribution No. 580, Technical Series, of the Texas Agricultural Experiment Station.

cinchonamine, and strychnine, the last being the most active. All of the above alkaloids produced a favorable action on alcoholic fermentation except cinchonamine and quinine.

*Botrytis cinerea* Pers. was cultivated by Nobecourt (10) on Raulins liquid containing varying amounts of nicotine and sulphates of atropine, quinine, and aconitine. He found that nicotine sulphate in a concentration of .025 M and atropine sulphate in a concentration of .020 M did not hinder the growth of this fungus. Growth was not retarded by .010 M of quinine sulphate, but a concentration of .020 M resulted in small thalli with few conidiophores, while one of .030 M prevented growth. Growth was visibly hindered by aconitine in a concentration of .002 M and greatly reduced by a concentration of .004 M. A concentration of .010 M did not prevent the germination of spores, which was completely inhibited at .020 M.

Smith (12) reported that *Botrytis cinerea* grew very poorly in 1 per cent solutions of brucine and strychnine and that no growth occurred in a 2 per cent solution of quinine and caffeine. Ravaz and Gouirand (11), however, found that this fungus grew normally at 0.1 per cent concentration. The effect of caffeine, quinine, and nicotine on the germination of *Botrytis cinerea* spores was studied by Staritzky (14). He found no germination at 0.1 per cent and normal germination at .001 per cent of caffeine; normal germination with .001 per cent quinine sulphate; no germination at .001 per cent, and normal germination at .0001 per cent of nicotine.

Yasuda (17) found that the growth of *Penicillium glaucum*, *Aspergillus niger*, *Botrytis cinerea*, and *Mucor stolonifer* was increased by the addition of the hydrochlorides of cocaine, quinine, cinchonine, morphine, codine, and strychnine to Richards nutrient solution. The alkaloids were used in concentrations varying from 0.2 to 2 per cent. As the concentration of alkaloid was increased, the conidiophores and sporangiophores became thinner and shorter. Formation of conidia and sporangia was entirely suppressed and replaced by that of chlamydospores when the optimum concentration for fungus-vegetation was surpassed. The weakest alkaloid for the fungi under consideration was the hydrochloride of morphine, while the strongest was that of cocaine. The fungi listed in order of their decreasing resistance to alkaloids are : *P. glaucum*, *A. niger*, *B. cinerea* and *M. stolonifer*.

Ehrlich (2) grew *Oidium lactis*, *Aspergillus niger*, *Penicillium glaucum*, *Willia anomala*, *Pichia farinosa*, a mixed culture obtained by exposing the culture solution to the air, and an unknown species of wine yeast on a mineral nutrient solution to which he added different alkaloids as the only source of nitrogen and ethyl alcohol or invert sugar as the source of carbon in concentrations of 0.2 and 2 per cent, respectively. A control series without the alkaloid was run at the same time. The alkaloids used at 0.2 per cent were : pyridine, piperidine, coniine, nicotine, cinchonine, quinine, brucine, cocaine and morphine. Ehrlich obtained only a small amount of growth, least in the yeast cultures and greatest in the mixed cultures. The retardation of growth he obtained was attributed to the poisonous action of the decomposition products of the alkaloids.

Enders and Wieninger (3) noted the effect of alkaloids on fermentation and multiplication of yeast. They found that the toxicity of alkaloids for yeast (as measured by the inhibition of fermentation and multiplication) diminished in the order: quinine, caffeine, cinchonine and pilocarpine. In general, much higher concentrations of alkaloids were required to inhibit the power to multiply than to inhibit fermentative ability.

#### MATERIALS AND METHODS

Certain of the alkaloids used in this investigation were secured from the following: R. H. Manske, National Research Council, Ottawa, Canada; James F. Couch, Bureau of Animal Industry, Washington, D. C.; W. M. Neal, Florida Agricultural Experiment Station, Gainesville, Florida; H. Kondo, Imperial University, Tokyo, Japan; J. Madinaveitia, University of Edinburgh, Edinburgh, Scotland; Karl Folkers, Merck and Co. Inc., Rahway, New Jersey. The courtesy of these investigators in providing alkaloids of their own isolation and purification is greatly appreciated. The alkaloids from *Mahonia*, *Berberis*, and *Sanguinaria* species were isolated and purified in this laboratory. The remainder were obtained from commercial sources and used without further purification.

The effect of the alkaloids on the growth of *Phymatotrichum omnivorum*, *Sclerotium rolfsii* Sacc., *Fusarium vasinfectum* Atk., *Verticillium albo-atrum* McA., *Rhizoctonia solani* Kühn, *Armillaria mellea* (Vahl.) Quel. and *Ophiobolus graminis* Sacc. was tested by growing the fungi on a liquid medium to which the alkaloid had been added in concentrations from .01 M to .0001 M. The nutrient solution<sup>3</sup> consisted of MgSO<sub>4</sub>, 0.75 g.; K<sub>2</sub>HPO<sub>4</sub>, 1.35 g.; NH<sub>4</sub>NO<sub>3</sub>, 1.00 g.; KCL, 0.15 g.; and dextrose, 40.00 g. per l. After removal of heavy metals by the method of Steinberg (13), Mn<sup>++</sup>, Fe<sup>++</sup>, Cu<sup>+</sup>, and Zn<sup>++</sup> were added at concentrations of 2.5 p.p.m. This solution is a modification of the nutrient solution 70 of Ezekiel, Taubenhaus, and Fudge (4) and has been shown by Talley and Blank (15) to give a proper supply and balance of the major and minor elements necessary for this organism. The pH of the final nutrient solution was approximately 6.5.

The necessary amount of each alkaloid was added to sterile 250-ml. Florence flasks. Usually this was done by pipetting the proper quantity of a 95 per cent ethyl-alcohol solution of the compound into the flask; sometimes, however, it was more convenient to weigh the alkaloid directly into the flask and add one ml. of alcohol for sterilization afterward. After the alcohol had evaporated, to each flask was added 25 ml. of sterile nutrient solution. Thus the opportunity for error due to heating chemicals and nutrient solution in presence of each other was avoided. The inoculum for each flask consisted of a disc 6 mm. in diameter cut from a nutrient agar plate covered with the mycelium of the fungus. The average dry weight of each disc was 2.5 to 3 mg. The cultures were incubated at 28° C. for the

<sup>3</sup> In one experiment with *Sclerotium rolfsii* and *Ophiobolus graminis*, potato dextrose medium was used in addition to the standard solutions reported.

following lengths of time shown by preliminary experiments to yield approximately maximum mat weights: *Sclerotium rolfsii*, 4 days; *Fusarium vasinfectum* and *Rhizoctonia solani*, 7 days; *Verticillium albo-atrum*, 10 days; *Phymatotrichum omnivorum* and *Ophiobolus graminis*, 21 days; *Armillaria mellea*, 28 days. After incubation, the fungus mats were removed by means of a hooked rod, washed with distilled water, dried to constant weight at 80° C. and weighed on an analytical balance. Each value reported is the average obtained from six cultures inoculated at three successive times, or, in a few instances, in triplicate at two different periods. The control values were obtained from six flasks without alkaloids run at each replication.

#### EXPERIMENTAL RESULTS

The results of growth studies on *Phymatotrichum omnivorum*, *Sclerotium rolfsii*, *Fusarium vasinfectum*, *Verticillium albo-atrum*, *Rhizoctonia solani*, *Armillaria mellea* and *Ophiobolus graminis* in nutrient solution to which alkaloids were added in concentrations of .01 M to .0001 M are presented in table 2. In table 1 are listed 62 alkaloids in order of their decreasing toxicity to *P. omnivorum* as measured by inhibition of mat weight. Although molar concentrations have been used, the p.p.m. for .0001 M concentrations are listed for convenience in making comparisons on this basis.

A study of the data in table 1 reveals the fact that certain alkaloids are highly toxic to *Phymatotrichum omnivorum* at low concentrations, while others are not inhibitory at high concentrations. At the lower concentrations of some of these compounds there are indications of a slight stimulation of growth over that of the controls. The results indicate that this fungus may use the nitrogen base xanthine as a source of nitrogen, since the yields were 468 mg., 437 mg., and 419 mg., respectively, as compared with 397 mg. for the controls, for the concentrations of .01 M, .001 M and .0001 M.

#### Toxicity of Alkaloids to Other Fungi

As indicated earlier in the paper, the study of certain of the alkaloids was extended to include *Sclerotium rolfsii*, *Ophiobolus graminis*, *Rhizoctonia solani*, *Armillaria mellea*, *Fusarium vasinfectum*, and *Verticillium albo-atrum*. These data are presented in table 2. The order of decreasing ability of these fungi to tolerate the alkaloids are: *V. albo-atrum*, 1.7<sup>4</sup>; *F. vasinfectum*, 2.0; *R. solani*, 3.5; *A. mellea*, 4.2; *O. graminis*, 4.8; *S. rolfsii*, 5.5; *P. omnivorum*, 6.3. The order of toxicity of the compounds to the 7 fungi are: sanguinarine 1.1, delphinine 2.4, berberine 4.1, gramine 4.3, solanine 4.3, veratrine 4.7.

#### DISCUSSION

Although a compound must contain nitrogen in a ring to be classified as an alkaloid, the other groups present exert a profound influence upon

<sup>4</sup> The values given are the average relative order of each of the 7 fungi in ability to tolerate the alkaloids. The values for alkaloids are the average order of each in toxicity to the fungi.

the physiological behavior of the molecule. Unfortunately, the compounds tested are so complex and the proportion of the total number of possible compounds so small that it is not possible at this time to make any broad generalizations regarding the relationship between toxicity and chemical or physical properties. Nevertheless it is of interest to note the results obtained with several groups of related compounds. For example, the addition of methyl groups to xanthine, to form theobromine (3, 7-dimethylxanthine) and caffeine (1, 3, 7-trimethylxanthine) increases progressively the toxicity to *Phymatotrichum omnivorum*. Hypaphorine, the methylbetaine of tryptophan, was found to inhibit greatly the growth of *P. omnivorum* at a concentration of .005 M. Brucine, dimethoxystrychnine, is more toxic than strychnine itself. The influence of methyl and methoxy groups attached to the benzene ring upon the growth of *P. omnivorum* is reported elsewhere (8).

All plants contain basic nitrogenous compounds, protein degradation products, choline, betaine, or similar substances that will react with certain of the alkaloidal reagents. However, the occurrence of alkaloids in the accepted use of this term is confined to a rather restricted number of botanical groups. Some of the large groups of plants do not so metabolize their nitrogen as to yield alkaloids. This is true of many of the genera of the Labiatae and Compositae, although there is one outstanding exception in the genus *Senecio* of the Compositae. The grasses are not characteristically alkaloid-bearing. Although recent studies have revealed that gramine is present in *Arundo donax* and *Hordeum vulgare* var. Chevalier I and II, Primus I and II, Gold x Chevalier, etc.<sup>5</sup>; lolaine in *Lolium temulentum*; an unidentified alkaloid in *Oryza sativa*. Alkaloids isolated from other monocotyledonous plants are lycorine from some twenty species of the Amaryllidaceae; veratrine from *Veratrum sabadilla*, *V. album*, *V. lobelianum*, *V. viride*, and *V. nigrum*. A number of the Liliaceae genera have yielded several alkaloids, e.g., *Fritillaria* and *Zygadenus*. The palms, with the exception of the areca or betel palm, are nonalkaloidal plants. In general, alkaloids are yielded by such important families as the Ranunculaceae, Berberidaceae, Papaveraceae, Fumariaceae, Leguminosae, and Solanaceae; these families contain species resistant to *Phymatotrichum* root rot.

These data (Table 1) furnish information on 50 to 70 species of plants from 15 families that differ in their susceptibility to *Phymatotrichum omnivorum*. The relative toxicity of these alkaloids to *P. omnivorum* follows in general the relative resistant rating of the plant from which they have been isolated. The correlation between the presence of alkaloids in plants and their resistance rating to *P. omnivorum* has been published (5).

The toxicity of alkaloids to this fungus does not correspond to their relative toxicities for the animal organism. A similar observation on the influ-

<sup>5</sup> Brandt, K., H. V. Euler, et al. Hoppe-Seyl. Zeitschr. Phys. Chem. 235: 37-42. 1935. They found that the presence of gramine was correlated with the resistance of barley varieties to nematodes. Hordenine also has been isolated from barley, and it is identical, according to Späth, with anhaline from *Anhalonium fissuratum* (Cactaceae).

TABLE 1.—*Influence of alkaloids on the growth of Phymatotrichum omnivorum*

Compound <sup>h</sup>	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					
	.01 M	.005 M	.001 M	.0005 M	.0001 M	P.p.m. of free base at .0001 M conc.
Sanguinarine <sup>a</sup>	0	0	0	0	0 <sup>i</sup>	35.1
Sanguinarine <sup>a</sup> SO <sub>4</sub>	0	0	0	0	0	35.1
Chelerythrine HCl <sup>a</sup>	0	0	0	0	0 <sup>i</sup>	41.1
Lycoerine <sup>b</sup>	0	0	0	0	28 <sup>j</sup>	28.7
Oxycaanthine <sup>a</sup>	0	0	0	139	(14-74) 208	31.1
Delphinine	0	0	0	155	(120-159) (185-256)	57.7
Berbamine <sup>a</sup>	0	0	0	162	(139-174) (221-402)	33.3
Berberine HCl <sup>a</sup>	0	0	0	268	(145-201) (214-341)	44.3
Quinine	0	0	29	211	(15-46) (193-231)	32.4
Veratrine	0	0	65	223	(65-223) (329-363)	59.1
Gramine (donaxine) <sup>c</sup>	0	28 (17-41)	158 (129-171)	241 (190-253)	213 (198-229)	17.2
Protopine <sup>d</sup>	0	75 (63-91)	138 (101-169)	300 (280-311)	350 (340-365)	35.3
Lobeline SO <sub>4</sub>	0	85 (67-103)	161 (136-190)	305 (293-319)	346 (311-397)	32.1
Spartiodined	0	99 (86-112)	172 (133-278)		334 (309-380)	33.3
Integerrimined <sup>d</sup>	0	102 (85-121)	186 (165-225)		339 (317-346)	33.5
Quinidine	0	109 (89-117)	295 (276-315)		369 (360-389)	36.9
Aspidospermine	0	115 (99-132)	233 (211-240)		364 (349-384)	35.4
Ephedrine	0	118 (97-137)	214 (183-255)		381 (374-386)	18.3
Cinchonine HCl	0	124 (110-134)	285 (169-330)		385 (368-421)	29.4
Nicotine	0	142 (107-159)	307 (240-394)		340 (315-360)	16.2
Scoulerined <sup>d</sup>	0	145 (123-169)	308 (291-326)		344 (336-351)	32.7
Caffeine	0	149 (139-163)	327 (240-388)		363 (300-401)	19.4
Hypaphorine <sup>e</sup>	0	151 (120-169)	314 (283-328)		360 (327-386)	28.2
Retrorsained <sup>d</sup>	0	154 (129-180)	310 (225-357)		347 (329-374)	35.1
Monocrotaline <sup>f</sup>	0	159 (139-182)	311 (287-377)		360 (336-407)	32.8

TABLE I.—(Continued)

Compound <sup>b</sup>	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					P.p.m. of free base at .0001 M cone.
	.01 M	.005 M	.001 M	.0005 M	.0001 M	
Lupinine <sup>c</sup>	0	160 (139-190)	311 (271-387)		374 (340-444)	35.2
Eserine (physostigmine)	0	163 (143-182)	315 (269-382)		370 (349-401)	27.5
Atropine	0	164 (134-192)	320 (301-352)		369 (326-435)	29.8
Sparteine SO <sub>4</sub> <sup>d</sup>	0	165 (136-194)	339 (309-387)		357 (339-386)	23.4
Pelletierine SO <sub>4</sub>	0	166 (146-187)	316 (277-366)		379 (350-406)	14.1
Corlumine <sup>d</sup>	0	198 (173-210)	322 (296-340)		382 (344-415)	38.3
Corydine HCl <sup>d</sup>	0	200 (183-215)	323 (296-344)		363 (329-398)	36.9
Deltoline <sup>e</sup>	0	298 (283-315)	365 (320-445)		384 (344-453)	39.6
Betaine HCl	0	303 (289-314)	374 (315-484)		419 (397-436)	11.7
Tryptamine	0	320 (300-359)	374 (341-400)		408 (389-421)	16.0
Brucine	4 (0-7)	333 (319-356)	349 (311-397)		389 (349-422)	46.6
Bicuculline <sup>d</sup>	7 (0-13)	333 (310-357)	372 (322-414)		361 (337-370)	36.7
dl-lupanine <sup>e</sup>	20 (6-70)	339 (310-360)	346 (318-400)		404 (376-450)	24.8
1-tetrahydro-palmatine <sup>d</sup>	42 (7-68)	340 (320-371)	314 (274-365)		353 (346-368)	35.5
Bicucine <sup>d</sup>	56 (37-67)	341 (316-351)	379 (297-416)		371 (329-404)	38.5
Ochotensine <sup>d</sup>	126 (102-137)	338 (327-342)	335 (323-340)		380 (369-391)	35.1
Strychnine HCl	137 (105-194)		339 (291-381)		363 (337-402)	33.4
Cocaine HCl	142 (132-150)		321 (311-327)		368 (349-418)	30.3
Procaine HCl	185 (173-211)		352 (338-399)		367 (346-394)	23.6
$\alpha$ -erythro-idine <sup>e</sup>	211 (183-248)		334 (287-365)		347 (294-364)	27.3
Trilupine <sup>e</sup>	242 (196-336)		357 (333-411)		401 (376-456)	31.6
Histamine di HCl	271 (258-294)		385 (343-419)		422 (411-439)	11.1
Tyramine HCl	273 (263-288)		364 (353-378)		429 (382-470)	13.7
Hyoscine HCl	288 (260-305)		351 (344-388)		361 (348-385)	30.3

TABLE 1.—(Continued)

Compound <sup>h</sup>	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					P.p.m. of free base at .0001 M cone.
	.01 M	.005 M	.001 M	.0005 M	.0001 M	
Theobromine	306 (296-318)		356 (349-360)		407 (375-455)	18.0
Choline HCl	322 (311-330)		387 (286-425)		389 (283-423)	12.1
Colchicine	331 (302-377)		373 (326-455)		406 (365-451)	39.9
Capaurined <sup>d</sup>	336 (304-374)		355 (341-370)		370 (360-385)	37.3
Capauridine <sup>d</sup>	337 (332-341)		354 (346-360)		353 (341-365)	37.3
Hyoscyamine HCl	339 (326-349)		372 (355-399)		358 (331-401)	28.9
Hydrastine	343 (323-356)		360 (329-397)		373 (336-419)	38.3
Homatropine	358 (339-382)		375 (337-407)		384 (341-411)	27.5
Aconitine	359 (310-392)		351 (322-374)		350 (337-360)	64.7
Adrenaline animal alkaloid	393 (369-416)		429 (401-457)		439 (430-448)	18.3
Xanthine	468 (450-473)	..	437 (420-446)		419 (410-435)	15.2
Controls = Av. 397 (346-452)						

<sup>a</sup> Isolated and purified in U.S.D.A. Plant Physiology Laboratory, College Station, Texas.

<sup>b</sup> Donated by Dr. H. Kondo.

<sup>c</sup> Purchased from Dr. J. Madinaveitia.

<sup>d</sup> Donated by Dr. R. H. Manske.

<sup>e</sup> Donated by Dr. Karl Folkers.

<sup>f</sup> Donated by Dr. W. N. Neal.

<sup>g</sup> Donated by Dr. James Couch.

<sup>h</sup> Solanine (glucoside-alkaloid) and zygadenum alkaloids were tested at 0.1%; .01%; and .001% and yielded 0 mg., 14 mg. and 354 mg.; 0 mg., 19 mg. and 252 mg., respectively.

<sup>i</sup> Sanguinarine was found to completely inhibit the growth of the fungus at 2.5 p.p.m.; chelerythrine tested under similar conditions yielded 3.4 mg. fungus mat at 10 p.p.m. (6).

<sup>j</sup> The first number given is the average value of 6 cultures, while the figures in parenthesis show the range of growth, i.e., the minimum and maximum values obtained.

ence of quinine, caffeine, cinchonine, and pilocarpine on the fermentation and multiplication of yeast has been recorded by Enders and Wieninger (3).

The data (Tables 1 and 2) of this investigation indicate clearly that it is prudent to reserve judgment on the protective rôle of an alkaloid until it has been isolated from the host tissue in the pure form. The concentration and localization, as well as the toxicity of the compound to the parasite in ques-

TABLE 2.—Influence of certain alkaloids on the growth of seven fungi. The results are calculated as the percentage of the controls

Dry weight (mg.) of fungi grown at various molar concentrations of compound, calculated as percentage of control																							
Organism <sup>a</sup>	Culture medium <sup>b</sup>	Incubation period days	Sanguinarine SO <sub>4</sub>			Berberine HCl			Veratrine			Gramine			Delphinine			Solanine					
			.01	.001	.0001	.01	.001	.0001	.01	.001	.0001	.01	.001	.0001	.01	.001	.0001						
N. S.r.	P.d.	4	160 <sup>c</sup>	0	0	0	0	0	3.8	11.3	22.5	55.6	0	26.9	41.3	0	22.5	45.6	0	6.9	59.4		
		70	11.5	0	0	0	0	0	0	0	39.1	90.4	0	31.3	53.0	0	29.6	76.5	0	15.7	95.4		
P.o.	P.d.	70	397	0	0	0	0	0	79.6	0	16.4	89.7	0	39.8	53.7	0	0	78.1	0	2.5	86.9		
O.g.	R.s.	21	108	0	0	0	0	0	3.7	56.5	90.7	0	76.9	86.1	0	69.4	81.5	0	33.3	84.3	0	0	85.2
A.m.	F.v.	70	496	0	0	4.8	13.5	73.8	99.0	34.3	94.2	99.2	0	93.8	99.8	0	3.8	96.6	0	70.6	99.0		
V.a.	V.a.	28	102	0	11.8	33.3	0	47.1	65.7	0	18.6	92.2	0	40.2	82.4	0	8.8	39.8	12.8	90.2	96.1		
		70	7	232	0	0	18.5	59.9	88.4	96.1	29.7	103.9	102.2	11.2	96.1	112.1	6.9	56.9	79.8	60.8	71.1	87.1	
		10	367	0	0	9.0	73.3	96.2	101.6	0	104.9	99.7	15.8	95.6	103.3	10.1	85.6	99.7	84.2	95.9	98.9		

<sup>a</sup> Abbreviations used in this table are: Sr. = *Sclerotium rolfsii*; Po. = *Phytophthora omnivorum*; Og. = *Ophiobolus graminis*; R.s. = *Rhizoctonia solani*; A.m. = *Armillaria mellea*; Fr. = *Fusarium vaginiforme*; Va. = *Verticillium albo-atrum*. R.s., Sr.r., and A.m. were secured from W. N. Eackiel; F.v. and V.a. from C. D. Shantz; C. C. A. = *Cylindrocarpon clavigerum*; Dc. = *Dactyliosphaera ciliata*; Nv. = *Nectriella violacea*; V. = *Valsa*.

C. B. Sherbakoff; U. S. from Hurley Fellows; 1-0, isolate No. 28 from L. M. Blank.

<sup>a</sup> P.d. = Potato dextrose; <sup>b</sup> No. 70 = standard solution described in text.  
<sup>c</sup> Since replicates within a treatment were average in uniformity, the range of mat weights was omitted.

tion, should be determined before predicting the possible relation of a given compound to the resistance or immunity of a plant.

#### SUMMARY

Sixty-two different alkaloids from 15 families and 50 to 70 species of plants differing in their susceptibility to *Phymatotrichum* root rot have been studied as to their influence on the growth of this fungus. Sanguinarine was found to be the most toxic alkaloid studied. It completely inhibited the growth of *Phymatotrichum omnivorum* at a concentration of 2.5 p.p.m. The alkaloids are listed in order of their decreasing toxicity, on a molar basis, to *P. omnivorum*: Sanguinarine, chelerythrine, lycoerine, oxyacanthine, delphinine, berbamine, berberine, quinine, veratrine, gramine, protopine, lobeline, spartiodine, integerrimine, quinidine, aspidospermine, ephedrine, cinchonine, nicotine, scoulerine, caffeine, hypaphorine, retrorsine, monocrotaline, lupinine, eserine, atropine, sparteine, pelletierine, corlumine, corydine, delta-line, betaine, tryptamine, brucine, bienculline, dl-jupanine, 1-tetrahydro-palmatine, bicucine, ochotensine, strychnine, cocaine, procaine,  $\alpha$ -erythro-idine, trilupine, histamine, tyramine, hyoscine, theobromine, choline, colchicine, capaurine, capauridine, hyosecyamine, hydrastine, homatropine, aconitine, adrenaline, xanthine.

The influence of 6 alkaloids on growth of *Phymatotrichum omnivorum*, *Sclerotium rolfsii*, *Ophiobolus graminis*, *Armillaria mellea*, *Rhizoctonia solani*, *Fusarium vasinfectum*, and *Verticillium albo-atrum* in liquid culture were studied, and it was found that the fungi show increasing ability to tolerate alkaloids generally in the order given. Although the fungi reacted differently to different alkaloids, the order of decreasing potency among the compounds tested was sanguinarine, delphinine, berberine, gramine and solanine, and veratrine.

In general, the relative toxicity of the alkaloids studied to *Phymatotrichum omnivorum* follow the relative resistant rating of the plant from which they were isolated. This indicates that certain alkaloids in roots of plants constitute an important factor in the resistance of these plants to *P. omnivorum*.

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## TOXICITY OF PARADICHLOROBENZENE IN RELATION TO CONTROL OF TOBACCO DOWNTY MILDEW<sup>1</sup>

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### INTRODUCTION

Experimentation involving the use of organic compounds to control tobacco downy mildew has now been in progress for several years. Empirical methods were used in our early experiments (3) with fumigants; but, gradually, a better understanding of the principles underlying their use has been evolved (5, 8). These studies have shown that volatile materials may be more effective fungicides than the nonvolatile ones ordinarily employed as dusts or sprays. It seems probable that the greater efficacy of volatile materials is related to their action not only as protectants but also as eradicants. This latter mode of action is novel in the field of plant pathology and is dependent upon the ability of volatile substances to penetrate infected leaves and either to inhibit the development of the pathogen or to be lethal to it, *in situ*, without apparent injury to the host tissues.

Although our studies have been concerned primarily with benzol, other volatile materials have been tested and found to have a similar mode of action. Prominent among those that have given promise of success is paradichlorobenzene, p-C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub>. This volatile crystalline product has long

<sup>1</sup> Cooperative investigations conducted by the Virginia Agricultural Experiment Station and Duke University.

been used as an insecticide, especially against peach-tree borers and clothes moths. Apparently, no attempts were made to employ paradichlorobenzene (hereafter called PDB) to control plant diseases prior to 1936, when it was used in seedbeds as a fumigant against downy mildew (3). In these experiments the crystals of PDB were placed in pans resting on the soil amongst infected plants, but without appreciable beneficial effect. Two years afterward the late W. M. Lunn, of Florence, South Carolina, observed that, in seedbed experiments, PDB had considerable promise as a control agent of this tobacco disease, and encouraged investigations by others with this material. Subsequently, reports by Clayton (1) and Pinckard and McLean (6) appeared. The most important results from the work of Pinckard and McLean (6) were that they called attention to failure to control downy mildew if ordinary seedbed covers are used for retaining PDB vapor within the bed, that best control occurs if the area of the surface on which the crystals are distributed to be vaporized is equal to that of the seedbeds, and that this compound becomes an eradicant fungicide if used in sufficient concentrations.

It appeared desirable to learn what concentration of PDB could be tolerated by tobacco seedlings on the one hand, and what strength was toxic to the tobacco downy-mildew fungus on the other. No methods were available by which this could be accomplished, since the usual methods of evaluating the fungicidal or germicidal properties of chemical substances are manifestly of little value when applied to gaseous fungicides. It became necessary, therefore, to devise, first of all, a laboratory method for testing the fungicidal value of PDB, to demonstrate the accuracy of this method, and finally to initiate a series of laboratory studies to determine the minimal concentration of this fumigant that can be safely used against tobacco downy mildew.

The present report embodies the results of these studies with PDB that are deemed basic to experimentation involving tobacco plants growing in seedbeds. In addition it discusses a general procedure applicable to investigation of volatile fungicides.

#### APPARATUS AND METHODS

Laboratory studies on the fungicidal value of volatile substances would appear to be most useful and the results would seem best to serve as the basis for subsequent field experiments if both the host and parasite could be acted upon simultaneously by the chemical under consideration. It would appear, furthermore, that an apparatus constructed to accomplish this purpose should possess the following features: It should be so constructed as (a) to insure a controlled concentration, in the air, of the vapors of the volatile substance to be tested; (b) to provide for continuous flow of a stream of the vapor-air mixture at a constant rate through chambers containing living seedlings; and (c) to maintain constant environmental conditions for the desired period of time. Since no such apparatus has

been described in the literature, one especially designed to meet the above requirements was constructed for the present studies.

A diagram of this apparatus is shown in figure 1. Air was introduced

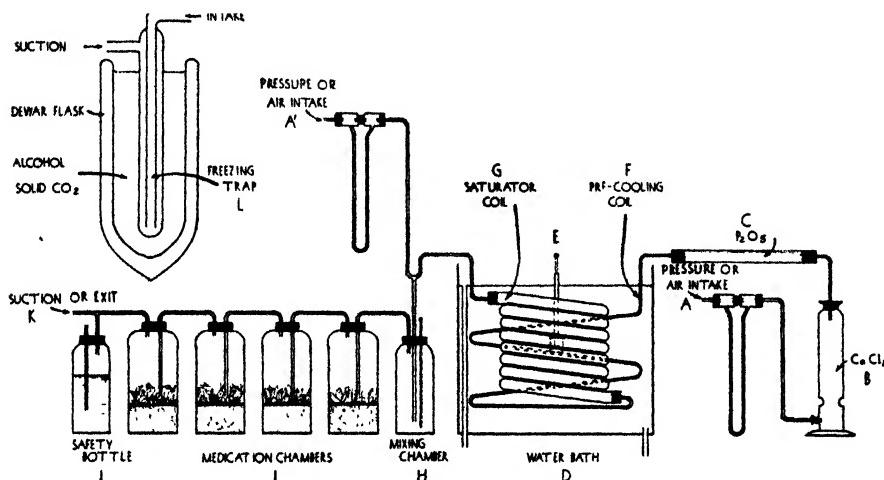


FIG. 1. Diagrammatic plan of apparatus employed to study toxicity of paradichlorobenzene.

into the apparatus through a calibrated flowmeter A. The air was partially dried by passage over calcium chloride B, and further desiccated over phosphorus pentoxide C, in order to prevent condensation of moisture in the saturator G and its subsequent accumulation in the absorption tubes or freezing traps L. In the experiments performed, using low concentrations of the vapor, the air was passed over concentrated sulphuric acid to assist in the removal of moisture. The dry air was then led into a copper tube of small bore coiled on the inside of the water bath D. This precooling coil F insured that air introduced into the saturator G would be of the proper temperature. Saturation of the incoming dry air was effected by leading it through a coil consisting of approximately 22 feet of  $\frac{1}{2}$  in. copper tubing that had been filled loosely with crystals of PDB of a size corresponding to the manufacturer's grade No. 6.<sup>2</sup> The vapor-air mixture, water bath, and coil were maintained at constant temperature during the course of the experiments. The temperature of the bath D was regulated by means of ice or was kept constant by flowing tap water. A milled copper block was soldered to the saturator coil G and a drill hole, with tube, was used to bring a thermometer bulb E in close contact with the wall of the saturator G. It is preferable to use two thermometers, one at each end of the saturator coil G. The air saturated with vapor was next led into a short copper tube submerged in the bath in order to prevent heat transfer from the atmosphere to the outlet of the saturator. The opening of this tube led into a Y tube, one

<sup>2</sup> The paradichlorobenzene employed, 99.8 per cent pure, was kindly supplied by the Solvay Sales Corporation, New York, N. Y.

arm of which was connected with a calibrated flowmeter with air intake A'. The other outlet of the Y tube led to a mixing chamber H, and the gas was drawn over the plants in chambers I similar to those described in an earlier paper (5). These chambers could be placed in another water bath, having either the same temperature or a higher one than water bath D. Maintenance at lower temperatures than those of water bath D would result in precipitation of crystals from the vapor if saturation conditions should prevail. To move the gas through the system pressure is preferred to suction. A slight positive pressure is preferable to negative pressure, as it eliminates possible dilution of the vapor stream of fixed concentration by leakage of air into the system. All connections were of the butt-type and were carefully protected with shellac, since PDB vapor is appreciably soluble in rubber.

#### ESTIMATION OF PDB VAPOR CONCENTRATION

The concentration of PDB vapor in the gas-air mixture, delivered to the chambers containing tobacco seedlings, was estimated by freezing out the paradichlorobenzene from a known volume of the vapor-air mixture. This was done by permitting a measured volume of the mixture to pass through suitable freezing traps. These traps (Fig. 1, L) were constructed of thin pyrex tubing with outside dimensions of approximately 120 by 10 mm., the inner tube being 4 mm. in diameter. After stoppering the side armus with corks and weighing they were put into the system, replacing the plant chambers I. A calcium chloride guard tube was connected with the exit arm of the freezing trap to prevent entry of moisture. After sweeping the air and moisture from the freezing trap, the flow of vapor-air mixture was stopped to permit the freezing trap to be lowered into a Dewar flask containing a freezing mixture of 95 per cent ethyl alcohol and solid carbon dioxide. The flow was then resumed and a known volume was drawn through the trap at a fixed rate. The temperature of the freezing mixture and trap was maintained at approximately -70° C.

Since the vapor pressure of PDB at -70° C. is negligible, the weight (*W*) in grams per liter of PDB frozen from the dry vapor-air mixture becomes a direct quantitative measure of the PDB content of the mixture being drawn through the system. Since the weight of the vapor and the volume of the gas, together with the temperature and the total pressure, were known, the partial pressure of the vapor was computed from the gas formula.<sup>3</sup>

<sup>3</sup> Where *W* is the weight of the vapor in g., *R* the gas constant in liter atmos., *T* the absolute temperature, *M* the molecular weight, *V* the total volume in liters, and *p* the partial pressure of the vapor in mm. then

$$p = \frac{W R T}{M V} \times 760$$

From the partial pressure *p*, and the total pressure *P* both in mm., the volume per cent of vapor present is computed by the relation

$$\text{volume per cent} = \frac{p}{P} \times 100$$

Since the total pressure *P* varied but slightly from the normal atmospheric pressure, *P* was regarded as 760 mm. in these computations.

A measure of the accuracy of the method of analysis of the PDB content of the vapor-air mixture is shown by the series of measurements in table 1. In series 4, 5, and 6 the saturated vapor-air stream was diluted with 7.5 l. of air, whereas saturation was maintained in all others.

TABLE 1.—*Analysis of PDB content for vapor-air mixtures delivered by apparatus for determining its fungicidal value*

Series	Temperature of vaporization	Weight of PDB per gross volume of gas-air mixture	Partial pressure		Concentration of PDB
			Calculated from weight	Calculated from vapor pressure-curve	
No.	°C.	G.	Mm.	Mm.	Vol. per cent
1	12.5-13.0	0.0418 <sup>a</sup>	0.317	0.312	0.041
2	13.0	0.0439 <sup>a</sup>	0.332	0.320	0.043
3	13.0	0.0415 <sup>a</sup>	0.314	0.320	0.041
4	13.5-14.0	0.0246 <sup>a</sup>	0.187	0.163 <sup>c</sup>	0.024
5	14.0	0.0250 <sup>a</sup>	0.190	0.166 <sup>c</sup>	0.025
6	13.5-14.0	0.0247 <sup>a</sup>	0.188	0.163 <sup>c</sup>	0.024
7	0.2	0.0168 <sup>b</sup>	0.078	0.090	0.0103
8	0.2	0.0176 <sup>b</sup>	0.082	0.090	0.0108
9	0.2	0.0177 <sup>b</sup>	0.082	0.090	0.0108

<sup>a</sup> 16 liters.

<sup>b</sup> 24.82 liters.

<sup>c</sup> Calculated from vapor pressure and dilution.

It becomes apparent by means of this method of analysis that a satisfactory approximation of the PDB content of a vapor-air mixture can be made. In this connection it may be pointed out that apparatus of the type just described should be generally useful in toximetric experimentation with other pathogens and with other volatile compounds.

#### DETERMINATION OF TOXIC AND FUNGICIDAL VALUE OF PDB

##### Methods

Following the development of apparatus capable of delivering a continuous stream of a vapor-air mixture at a constant PDB concentration, a series of experiments were undertaken to determine the maximal concentration of the fumigant tolerated by tobacco seedlings and the minimal concentration that is fungicidal. These experiments involved 1, varying the concentrations of PDB vapors and 2, varying the duration of exposure. The concentrations were varied by maintaining desired constant temperatures for saturating the air passing over the seedlings in the jars, or by diluting the vapor-saturated air mixture with a known volume of air introduced through the flow-meter A' (Fig. 1). After preliminary experiments involving exposures of 3, 6, or 9 hours, twelve-hour periods of exposure were selected. Applications were made at night. Each successive application was made after an interval of 12 hours.

The tobacco seedlings used included the varieties Yellow Mammoth, White Stem Orinoco, and Jamaica, all of which are equally susceptible to infection with downy mildew. They were grown in half-gallon, screw-cap,

glass jars containing approximately 500 g. of soil. When the seedlings were about 4 in. tall, the inoculum, consisting of a water suspension of freshly formed sporangia, was applied with a compressed-air atomizer. Conditions favorable for infection were then provided. Three or 4 days after inoculation the first application of PDB vapor was made, employing 3 to 5 jars of seedlings with each series. After each treatment the jars were aerated by directing a stream of air through the inlets until odor of PDB could no longer be detected. Absence of odor indicated that the concentration of PDB in the moisture present was less than 1 part per 100,000. This figure is approximately the lower limit of PDB concentration in aqueous solutions that gives a perceptible odor. It was estimated by the use of a Zeiss water interferometer to determine the amount of PDB in water solutions in which odor could just be detected. Experience showed that the jars required continuous aeration for several days to remove all traces of odor.

Two types of checks or controls were used, (a) inoculated nontreated seedlings and (b) noninoculated, nontreated seedlings. Fungicidal action following treatment with PDB was not apparent earlier than 6 to 8 days after inoculation, since this period corresponds with the length of the sporangial cycle. Toxicity to the host, however, was apparent either during the period of exposure or shortly thereafter.

The data resultant from these series involved seedlings in 183 jars, 131 of which were fumigated, while 42 served as inoculated controls and 10 served as healthy controls.

#### EXPERIMENTAL RESULTS

##### General Results

It was anticipated, as has been indicated, that the two factors (a) concentration of vapors and (b) duration of exposure to these vapors should be of primary importance, and, therefore, should be given major consideration in studying the toxicity of PDB. These factors would appear to be evaluated most succinctly by presenting certain selected data from among the body of data that has been secured. All other factors seem to be secondary, but it has not been possible quantitatively to assay the influence of each of these factors. Among the secondary factors that have been considered are mode by which tolerance of host and pathogen to PDB vapors is influenced by temperature and by presence of films of moisture on the foliage. Comparisons also have been made of tolerance of infected and healthy seedlings to PDB. It is clear from supplementary evidence that the above factors do not affect our results on the toxic limits within the precision of our determinations. Therefore, the following conclusions appear to be warranted. (a) The temperature at which seedlings are maintained during treatment, within the range 13° C. to 25° C. is without significant influence on the toxic and fungicidal limits. (b) The presence or absence of visible films of moisture on the foliage during fumigation does not appear to modify susceptibility of tobacco seedlings or of the pathogen to injury. (c) Infected

seedlings seem to be neither more nor less susceptible to injury than do healthy ones.

#### Influence of Varying Concentration of PDB

In contrast to the relative unimportance of the secondary variables just discussed is the influence of concentration and duration of exposure in determining toxic limits. The effect of concentration is clearly brought out by data in table 2, which apply to single applications.

TABLE 2.—*Fungicidal and toxic influence of varying concentrations of PDB. Single applications of 12 hours' duration*

Series No.	Total No. of jars used	Concentration of PDB vapors <i>Vol. per cent</i>	Control of pathogen, No. positive or negative	Injury to host
1	11	0.01	Negative, 11	None
2	4	0.014	Negative, 4	None
3	4	0.017	Negative, 4	None
4	14	0.02	Positive, 12 Negative, 2	None
5	4	0.022	Positive, 2 Negative, 2	None
6	3	0.0375	Positive, 3	Slight
7	7	0.042	Positive, 7	Slight, 6 Severe, 1

The data in table 2 indicate that when single fumigations of 12 hours duration are given, there is a range of concentration of PDB vapors within which fungicidal action against tobacco downy mildew does not occur. With increased concentration, however, there is a range in which sporangial formation is inhibited without evidence of injury to the tobacco seedlings. As the volume-percentage concentrations are further increased the seedlings are injured, least damage occurring with the lower concentrations. The termini of these ranges are not sharply delimited, an observation entirely in accord with similar studies involving biological materials. PDB is slightly fungicidal if infected plants are exposed for a 12-hour period to an atmosphere saturated with PDB vapors within the range of 0.01 to 0.022 volume percentage (equivalent to saturation at 0° C. to 7° C.), but the pathogen is not eradicated. Eradicant action was exhibited at complete saturation within the range 0.022 to 0.0375 volume percentage, equivalent to saturation at 7° C. to 12° C. Definite injury to seedlings resulted if the concentration was above 0.037 volume percentage. This is equivalent to saturation at 12° C.

#### Influence of Repeated Applications

It seemed probable that volume-percentage concentrations within the range found to be nonfungicidal with a single application of PDB might be effective if applications were repeated on successive nights. The importance of repeated applications is clearly evident from the data in table 3.

As was anticipated, repeated applications of PDB were found to be more

TABLE 3.—*Fungicidal and toxic influence of repeated applications of PDB at varying concentrations*

Series No.	Total No. of jars used	Duration of fumigation	Concentration of PDB vapors	Control of pathogen, No. positive or negative	Injury to host
		<i>Hr.</i>	<i>Vol. per cent</i>		
1	8	12	0.01	Positive, 2 Negative, 6	None
2	8	24	0.01	Positive, 6 Negative, 2	None
3	8	36	0.01	Positive, 6 Negative, 2	None
4	8	48	0.01	Positive, 8 Negative, 0	None
5	4	12	0.02	Positive, 2 Negative, 2	None
6	4	24	0.02	Positive, 4 Negative, 0	None
7	4	36	0.02	Positive, 3 Negative, 1	None
8	4	48	0.02	Positive, 4 Negative, 0	None
9	4	12	0.022	Positive, 2 Negative, 2	None
10	5	24	0.022	Positive, 5 Negative, 0	None
11	6	36	0.022	Positive, 6 Negative, 0	None
12	5	36	0.0467	Positive, 5 Negative, 0	Severe
13	5	48	0.0467	Positive, 5 Negative, 0	Severe

effective than a single application. The data indicate that within the range 0.01 to 0.02 volume-percentage concentrations, effective fungicidal action follows the use of 4 applications on consecutive nights. Three consecutive fumigations were required at a concentration of 0.022 and caused no injury to the plants. Concentrations of 0.046 used on 3 or 4 successive nights, however, caused severe injury to the seedlings.

#### GENERAL CONSIDERATIONS

The experimental results with PDB illustrate the principles that have already been set forth concerning the mode of action of volatile fungicides (8). An appreciation of the mode of action of volatile fungicides may be had if certain fundamental physical and chemical facts are borne in mind. Volatile chemicals are distributed over both the external and the internal surfaces of the leaves by virtue of their ability to evaporate at ordinary temperatures. By virtue of their solubility in water they enter into solution in external aqueous films, and in moisture within the cell where they may react with cell constituents. The concentration of vapors in water is determined (a) by the partial vapor pressure of the volatile chemical in the atmosphere in contact with aqueous films, and (b) by temperature. Fungicidal action depends upon the two factors, volume-percentage concentration and duration of treatment. While the fungicidal value of any given volatile

compound is related to each of the factors enumerated, there is also a specific effect. This depends on the differential interaction of the compound with host and pathogen, respectively. It may be either physical, involving effects such as solvent or surface action, or it may be a specific chemical interaction with particular cell constituents.

As concerns PDB there is a relationship between temperature, vapor pressure, volume-percentage concentration and the ranges within which injury to tobacco seedlings and to *Peronospora tabacina* occurs. This can best be appreciated if presented graphically, as in figure 2. This graph is based on findings in the present study and on PDB vapor-pressure measurements made in our laboratories and reported elsewhere (2). In it vapor pressure and the corresponding saturation vapor concentration are plotted as functions of the temperature. The limitation imposed by temperature

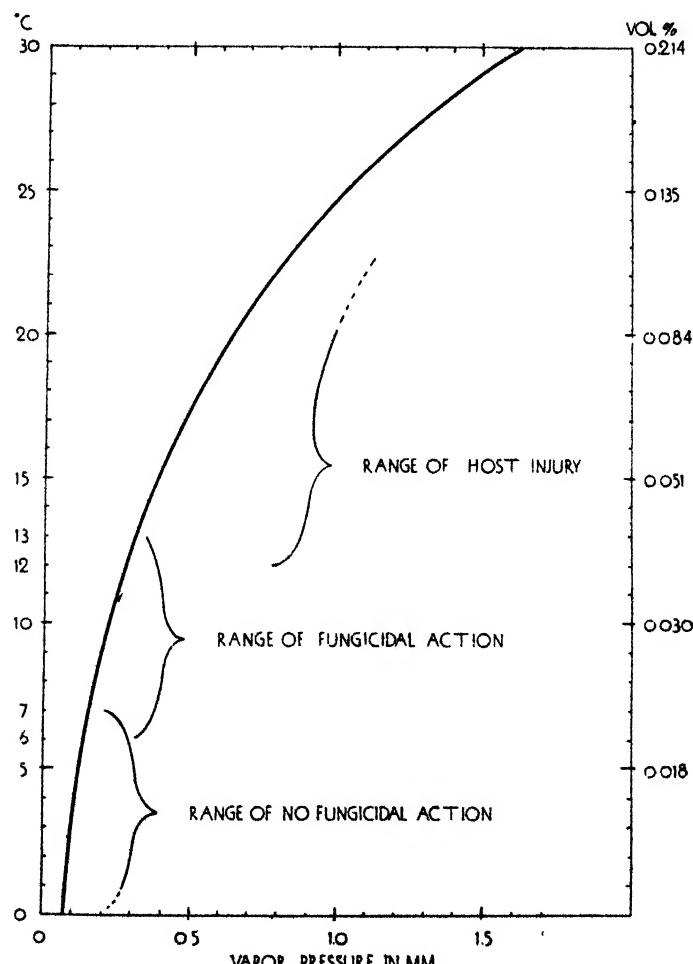


FIG. 2. Graph showing relationship of temperature, vapor pressure and volume percentage concentration of paradichlorobenzene at saturation and their ranges of toxic action in one 12-hour period.

on the vaporization of PDB is at once apparent. In the lower ranges the vapor pressures and, therefore, the saturation concentrations increase very slowly with temperature.

#### PRACTICAL IMPLICATIONS

While it has been possible by repeated fumigation to control tobacco downy mildew under laboratory conditions with saturation vapor pressures of PDB within the temperature range 0° to 7° C., this compound might be ineffectual in seedbeds within this temperature range for the reason that it would be impossible, because of leakage, to secure and maintain these saturation pressures. This limitation in use of PDB as a fumigant against tobacco downy mildew in seedbeds may be circumvented by certain procedures, as will be considered in an accompanying report (4) that details the results of field experiments.

#### HOST-PENETRANT FUMIGANTS

In view of the novel mode of action of PDB and related volatile substances, it seems desirable to designate them as host-penetrant fumigants. This emphasizes the important distinction that arises because of their ability to penetrate within tissues and act therein. This is in contrast to the immobility and lack of host-penetrating power of such agents as sprays and dusts that function as protectants on the external leaf surfaces.

It is clear that these penetrants could act in a number of different ways. In the cases of benzol and of PDB this action, which, either directly or indirectly, involves the host or the pathogen, is so effective that these compounds act as eradicants. The importance of such penetrant action is apparent when it is realized that it is possible by means of volatile fungicides to check the course of infection. In this sense they may be said to serve as curatives.

#### SUMMARY

Paradichlorobenzene, a volatile crystalline compound, is fungicidal to *Peronospora tabacina* and acts as an eradicant without appreciable injury to the host.

The minimal concentration of paradichlorobenzene vapor fungicidal to tobacco downy mildew is within the range 0.01 and 0.02 volume percentage, equivalent to saturation pressures within the temperature range 0° C. to 7° C. Three to 4 consecutive treatments within this range are requisite for effective fungicidal action. A single application within this range does not effect eradication.

The maximal concentration of paradichlorobenzene vapor tolerated by tobacco seedlings for a single 12-hour fumigation is approximately 0.0375 volume percentage, equivalent to saturation at 12° C.

Temperature also is a factor of major importance in delimiting the concentration of PDB vapor obtainable.

The toxic limits of PDB were determined by an apparatus that should be generally useful in toximetric experimentation.

The concept of penetrant fumigants is developed and its implications are indicated.

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### THE USE OF PARADICHLOROBENZENE IN SEEDBEDS TO CONTROL TOBACCO DOWNY MILDEW<sup>1,2</sup>

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#### INTRODUCTION

Field experiments involving the employment of paradichlorobenzene ( $p - C_6H_4Cl_2$ ) as a fungicide for preventing and controlling downy mildew on tobacco seedlings are here reported. In planning these experiments use was made of the results of field studies, performed in 1938, and of laboratory studies (2) performed during the current season. Only a limited number of field experiments with this volatile crystalline product had been performed prior to the current year. The laboratory studies have dealt with such fundamental problems as rate of vaporization in relation to size of crystals and to temperature, with methods for analysis of paradichlorobenzene in vapor-air mixtures, with measurements of the vapor pressure of this compound (1, 2), and with determinations of the minimal concentration of vapor that is fungicidal to the downy mildew fungus, and that which is toxic to tobacco seedlings. As might be anticipated, previous experiences with benzol as a fungicide were found valuable in the present studies.

The purpose of the present work was (A) to determine whether paradichlorobenzene (for brevity, hereafter designated as PDB) constitutes a de-

<sup>1</sup> Cooperative investigations conducted by the Virginia Agricultural Experiment Station and Duke University.

<sup>2</sup> Special thanks are due Mr. E. G. Moss, Tobacco Experiment Station, Oxford, N. C., for his whole-hearted cooperation.

pendable fungicide against tobacco downy mildew, and (B) to evaluate the modificatory influence of certain mechanical and environmental factors as (a) PDB vapor concentration within seedbeds, (b) temperature, (c) moisture conditions, (d) distribution of crystals, (e) amount of fumigant, (f) tightness of seedbed frames and covers, (g) volume of seedbed, (h) size of crystals and (i) interval between successive applications. A knowledge of these interrelated factors should be of value in seedbed practices. Not all of them, however, have been isolated and evaluated, but a body of experimental evidence applying to the use of PDB has been secured that contributes to an understanding of this problem.

#### METHODS AND MATERIALS

Three locations were selected in which to conduct these experiments, one near McDonald, North Carolina, representative of a poorly drained area of the lower Coastal Plain; another near Oxford, North Carolina, representative of the upper Coastal Plain; and the third near Chatham, Virginia, representative of the Piedmont area. By taking advantage of seasonal differences existing in these 3 localities, the duration of the period for making observations was extended over more than 2 months.

Weather conditions during 1939 were considered only moderately favorable for the development of the disease in each of the selected localities. Sporulation was first observed in the experimental seedbeds near McDonald on March 24, near Oxford on April 15, and near Chatham on April 28. Within each locality the severity of downy mildew varied greatly, depending upon soil-moisture conditions, air movement, and proximity to woods.

The seedbeds were framed with boards and divided into convenient compartments. Some of the beds were made by growers, and were either not framed or framed with logs. The covers were made to fit tightly, and, except in 2 series of tests involving the influence of covers of different textures, consisted of unbleached sheeting having 64 warp threads and 64 woof threads per inch, 3.5 sq. yd. of which were required to weigh one pound.

A technical grade of PDB, 99.8 per cent pure, was used.<sup>3</sup> The crystals of PDB were distributed either on shelves constructed of boards or on wire screens arranged along the sides near the top of the framing. Alternatively the crystals were broadcast over the top of the ordinary loose-texture seedbed cover (Fig. 1, A). The denser heavier covers were, of course, drawn over the ordinary cover after the crystals had been distributed.

Applications were made between 6 and 7 o'clock p. m., and the heavy covers were removed approximately 12 hours later.

Sampling the atmosphere within the seedbeds to determine its PDB vapor content was accomplished by means of a specially constructed aspirator (Fig. 1, B). This necessitated the installation of copper tubes at selected positions so that the inlet was inside the bed and the aspirator could be attached to the outlet. Aspiration was effected by displacement of water.

<sup>3</sup> The sizes of the crystals employed were those designated by the manufacturers, The Solvay Sales Corporation, as grades No. 1, 6, and 9.

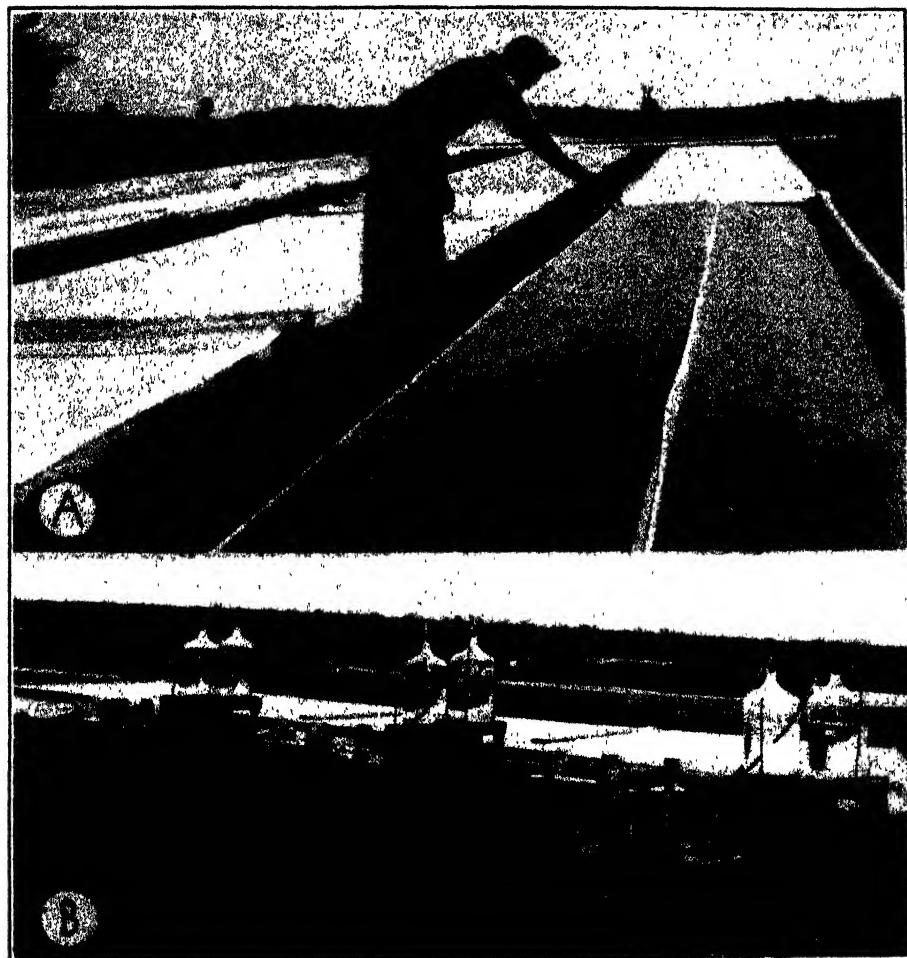


FIG. 1. A. Method of broadcasting crystals of paradichlorobenzene on ordinary seedbed cover. The heavy cover appears rolled back on bed in foreground. B. Aspirators used in sampling vapor-air mixtures in seedbeds.

The vapor-air mixtures were partly dried by passage over NaOH flakes. The PDB was removed from the vapor-air mixture by freezing traps, as previously described (2). From the weight of PDB crystals in the traps, the volume-percentage concentration of vapors in the atmosphere of the seedbeds was calculated (2).

#### EXPERIMENTAL RESULTS

##### Prevention and Control of Downy Mildew in Seedbeds

Tests of the efficacy of PDB were designed to determine whether this compound can be employed both to prevent and to control downy mildew in each of the 3 selected localities. Preventive applications were begun prior to the appearance of disease in the experimental beds, while applica-

tions to control were begun immediately after sporulation first became evident. Forty-seven seedbeds, ranging in size from 4 to 200 sq. yd. and having a total area of 1720 sq. yd., were employed. The treatments were successful in 36 of these beds and failed in the remaining 11 beds. Treatments were regarded as successful if further sporangial formation was completely inhibited or if sporangia formed on only 1 or 2 leaves throughout the entire period of treatment. The total area of successfully treated beds was 1612 sq. yd., and of unsuccessfully treated areas, 108 sq. yd. In the case of the successful treatments, PDB was applied every night in beds having a total area of 372 sq. yd.; and a total area of 1240 sq. yd. of seedbeds was treated at successive intervals greater than one night. Of the beds in which PDB failed to give satisfactory control 80 sq. yd. were treated every night and 28 sq. yd. were treated at successive intervals greater than one night. Consideration will be given subsequently to factors that determine whether treatments with PDB will successfully prevent or control downy mildew of tobacco or fail to do so. Suffice it to conclude that successful control was accomplished in each of 3 localities during a season when the disease was moderately severe, both by nightly application of PDB and by employing longer intervals between successive applications.

#### MODIFYING FACTORS

A proper understanding and interpretation of the results of experimentation with PDB as a fungicide against tobacco downy mildew necessitated that account be taken of the influence of several obvious factors. Primarily, these included the effect of the application of varying quantities of the chemical, and the effects of moisture and temperature conditions, in so far as they modify the evaporation of the crystals and the absorption of the fungicidal vapors. Consideration was given also to certain other factors that, although important, appear to be secondary. These include the mechanics for best distribution of crystals to insure the most efficient fungicidal action, variation in the frequency of applications, and the influence of size of crystals and of porosity of covers. In pointing out the influence of any single factor, in the accounts that follow, detailed statements concerning all other factors are omitted in order to avoid repetition. Such factors were either identical or were kept as nearly constant as is possible in experimentation involving seedbeds.

In a previous study (3) use was made of measurements of the volume-percentage concentrations of the benzol vapors present within the seedbed atmosphere throughout the night to evaluate certain factors governing the effectiveness of benzol, applied to tobacco seedbeds, in controlling downy mildew. Measurements were, therefore, instituted in the present studies with PDB to aid in evaluating the factors that contribute to proper use of this fungicide. Since measurements of volume-percentage concentrations of PDB in vapor-air mixtures by means of freezing traps are both arduous and time consuming, only a limited number were made.

### Influence of Varying Quantities of PDB

In this series of experiments measurements were made of concentrations of PDB vapors in the atmosphere of three seedbeds that were similar in every other respect, except that the equivalent of 1, 2, and 3 pounds of fumigant respectively per 100 sq. yd. of seedbed area was distributed over the surface of the tobacco seedbed covers. The heavier covers were then placed over them. The night remained continuously warm and there was no appreciable formation of dew. The essential conditions of this experiment and the results of the measurements are assembled in table 1.

TABLE 1.—*Influence of the application of varying quantities of PDB on the concentration of vapors in the air of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper-ature	Time elapsed after appli-cation	Concentration of PDB vapors in atmosphere	Moisture conditions
<i>Lb.</i>	<i>°C.</i>	<i>Hr.</i>	<i>Vol. per cent</i>	
1	16	5	.0025	Covers and foliage dry
1	15	8	.0009	do
1	17	11½	.0013	do
2	16	5	.0037	do
2	15	8	.0018	do
2	17	11½	.0014	do
3	16	5	.0046	do
3	15	8	.0025	do
3	17	11½	.0016	do

These data show that as the quantity of crystals applied per unit area of seedbed increases, the volume-percentage concentration of the vapor in the atmosphere also increases.

### Influence of Moisture

Comparative measurements of volume-percentage vapor concentrations of PDB in the atmosphere of seedbeds were made under identical conditions, except that the seedlings and the covers were sprinkled to keep them thoroughly wet in one case; in the other, the foliage and covers remained rather dry. Representative contrasting measurements of the vapor concentrations found are shown in table 2.

As was anticipated, markedly higher concentrations of vapors occur in

TABLE 2.—*Influence of moisture conditions upon concentration of vapors of PDB in the atmosphere of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper-ature	Time-elapsed after apply-ing PDB	Concentration of PDB vapors in atmosphere	Moisture conditions
<i>Lb.</i>	<i>°C.</i>	<i>Hr.</i>	<i>Vol. per cent</i>	
4.0	14.4	3.0	.0094	Foliage and covers dry
4.0	14.0	5.5	.0094	Foliage and covers dry
4.0	14.4	3.0	.0170	Foliage and covers wet
4.0	14.0	5.5	.0170	Foliage and covers wet

seedbeds when covers and foliage remain wet throughout the night. Evidently, the covers greatly retard loss of PDB vapors if they are wet, whereas they are much less effective in this respect if kept dry. This observation accords with previous findings relative to the influence of moisture on the covers in retarding the escape of benzol vapors (3).

#### Influence of Temperature

In data previously presented (2) the relationship between temperature and vapor pressure of PDB is clearly shown. From these data one would anticipate finding that differences in volume-percentage concentration of vapors should be correlated with differences in temperature. Measurements of vapor concentrations involving 2 contrasting temperature conditions are shown by the 2 following cases. In the first instance the equivalent of 3 pounds of crystals per 100 sq. yd. of seedbed area was applied in the daytime to the selected seedbed. The crystals were scattered upon the surface of the ordinary cloth, the heavy cover was drawn into position and wetted. Two hours after fumigation was begun, the temperature within the seedbed being 30° C., the volume-percentage concentration of PDB vapors was 0.0114. In another bed similarly treated, but in which the temperature was 19.5° C., the volume percentage concentration of PDB vapor was 0.0039, when measured 3 hours after fumigation was begun.

The influence of temperature also was approximated by collecting and weighing the crystals that remained in the morning. On nights when temperatures were 7° C. or lower, two-thirds of the crystals remained unvaporized in beds where the equivalent of 1.5 lb. of fumigant per 100 sq. yd. was used.

Insufficient vaporization occasioned by low temperature may result in failure to control tobacco downy mildew, under the limitations imposed by application in seedbeds. Such a condition was encountered during the past season in a cold, rainy period extending from the late afternoon of May 1 to mid-forenoon of May 4. The temperature dropped suddenly, beginning about 6 p. m. on May 1, at which time 4 lb. of PDB crystals were applied to a seedbed of 110 sq. yd. Within 3 hours the temperature had fallen to below 50° F. and, thereafter, remained continuously between 50° F. and 40° F. for 36 hours. Limited evaporation of crystals occurred during the first night and the heavy cover was allowed to remain undisturbed throughout the following day, since it was continuously cold and rainy. Sporulation was abundant on the morning of both May 2 and 3. A portion of the crystals still remained unvaporized on the latter morning when the bed was uncovered. The temperature during the day of May 3 rose to between 50° and 60° F., but the sky remained overcast. Another application of 4 lb. of PDB crystals was made during that afternoon. The temperature again fell to below 50° F. by 8:00 p. m., and gradually became colder throughout the night. Sporulation was again profuse on the morning of May 4, when it was found that an abundance of the PDB crystals still remained unvapor-

ized. Control in this instance was obtained by allowing the cover to remain undisturbed during the forenoon of May 4. The day was warm and sunny so the rise in temperature increased vaporization.

#### Influence of Distance from Source of PDB

PDB vapors are approximately 5 times heavier than air and, therefore, they could be expected to diffuse slowly throughout seedbeds, since the rate of diffusion of a vapor is inversely proportional to its density. The data in table 3 indicate the differences that can exist in distribution of PDB vapors as related to distance from source.

TABLE 3.—*Influence of distance from source of PDB upon concentration of vapors within the air of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper-ature	Time elapsed after appli-cation	Concentration of PDB vapors in atmosphere	Source of samples
Lb.	°C.	Hr.	Vol. per cent	
3.0	13.0	3	.0055	Sampled near source
3.0	10.4	6	.0092	do
3.0	10.0	8	.0077	do
3.0	10.0	10	.0081	do
3.0	13.0	3	.0020	Sampled at soil surface
3.0	10.4	6	.0031	do
3.0	10.0	8	.0035	do
3.0	10.0	10	.0034	do

The greater concentration of vapors may be noted to occur in the air near the crystals. Probably other factors, as absorption by moisture on the foliage and in the soil, were of more importance in determining the distribution of vapors within the bed than diffusion.

#### Amount of Paradichlorobenzene

Evidence from experience of the previous year had indicated that PDB, applied nightly at the rate of 1.5 lb. per treatment per seedbed area of 100 sq. yd., is sufficient to be effective in the control of tobacco downy mildew. In the present experiments the quantities applied were equivalent to 1, 1.5, 2, 3, or 4 lb. nightly.

In certain series, fumigation was initiated before outbreak of downy mildew; in others, treatments were applied only after infection was apparent. In any case satisfactory control resulted from the use of each of these quantities of PDB, under each set of conditions, when the fumigant was broadcast. Injury to the seedlings, manifest in yellowing of foliage and bleaching of tips of the older leaves, was apparent in beds that received the larger amounts. Most pronounced injury occurred if the temperature at night ranged from 20° to 25° C. In no case did injury to the host result from the use of 1 or 1.5 lb. per seedbed area of 100 sq. yd.

#### Size of Paradichlorobenzene Crystals

A study of comparative rates of evaporation of crystals of different sizes shows that the rate increased with decrease in size of crystals (1). Attempts

to take advantage of the slow rate of evaporation of large crystals (No. 1 size) to maintain low vapor concentrations in the beds, both night and day, were unsuccessful. In subsequent experimentation only smaller crystals of sizes 6 and 9 were employed in order to obtain as complete and as rapid vaporization as possible during the time available for each treatment. Both of these sizes, under comparable conditions, gave satisfactory control. Crystals of size 6 are preferred, however, because those of size 9 tend to cake, when applied on shelves, and to sift through when applied on the top of seedbed covers.

#### Distribution of Paradichlorobenzene

As has previously been stated, 2 methods were employed to distribute the crystals of PDB. One method involved shelves, either of boards or of screen-wire, arranged inside the bed along 2 sides, along 1 side, along the center, or some other modification of this arrangement. The other method involved broadcasting the crystals over the top of the thin cover in order that the area of distribution might equal that of the seedbed. Although satisfactory control followed the use of each method, failures resulted in some cases when the crystals were scattered on shelves. These failures appeared to be related to placement, distribution, and area of the shelves; but data are insufficient to show what arrangement of shelves would be satisfactory under all conditions. The best procedure appears to be to scatter the crystals over the top of the ordinary seedbed cloth and then cover this over with the heavier cloth.

#### Tightness of Seedbeds

Type of seedbed cover is an important consideration when employing volatile materials in the control of downy mildew. It may be recalled that there was lack of accord among the results of experiments performed in 1938, as to the type of seedbed cover required for successful fumigation with PDB. Accordingly, 1.5 lb. of crystals per application of 100 sq. yd. of seedbed area were employed, using 5 types of cloth with specifications as follows: (A) warp 24, woof 28, 14 sq. yd. to weigh 1 lb.; (B) warp 48, woof 48, 7 sq. yd. to weigh 1 lb.; (C) warp 48, woof 48, 4 sq. yd. to weigh 1 lb.; (D) warp 56, woof 60, 4 sq. yd. to weigh 1 lb.; (E) warp 64, woof 64, 3.5 sq. yd. to weigh 1 lb. The first and second types of cover correspond closely in density of weave to open-mesh and close-mesh tobacco-seedbed covers of the kinds ordinarily employed on seedbeds. They were given a careful trial because of statements to the effect that control of downy mildew could be obtained from the use of PDB, even with the ordinary seedbed covers. No apparent control resulted with the use of the first type of cloth in either of the two beds, the one treated every night, the other on alternate nights. Complete lack of control occurred on each bed, treated every night, when covered with the second type of cloth. Control was entirely satisfactory in each of the beds covered with the other 3 types of cloth. These results indicate that the covers must be sufficiently densely woven to become vapor-tight when

moistened, and that loosely woven covers possess little or no ability to retain vapors of PDB within the beds.

Type of framing constitutes a second consideration in making seedbeds sufficiently tight for effective fumigation. Boards can be properly joined to insure tightness. It is more difficult to make the seedbeds tight if log frames are used, but such beds, 200 sq. yd. in area, have been successfully treated when the crystals were applied upon the thin cover and if the heavy cover drawn over the top is sufficiently large to permit the lap to be firmly secured close to the ground outside of the frame.

#### Volume of the Seedbed

Emphasis has hitherto been placed upon surface area of the seedbed in determining the quantity of fumigant to apply. Manifestly, total volume as affected by depth of the seedbed is also a factor that should be taken into consideration. Our experiments have not involved the comparison of beds with frames of different heights. The depth of the beds used has been quite uniform throughout, and since the heavy covers were normally fitted tightly over the frames, the depth of the beds corresponded with the height of the frames. In certain cases however, the volume was decreased by allowing the heavy cover to sag down in the center of the bed. As a result of this decrease in volume the PDB vapor concentration within the bed was increased, as was anticipated. These observations suggest that fungicidal activity may be modified, if all other factors are constant, by elevating or lowering the heavy cover, and thus varying the volume of the seedbed.

#### Interval between Treatments

The apparent eradicant action of PDB suggested that fumigation might be effective if the interval between successive applications was longer than 1 night. Accordingly, experiments involving seedbeds at Oxford and Chat-ham were planned and treatments were made on alternate nights, at intervals of 3 nights, and at intervals of 4 nights between successive applications. The PDB was applied in amounts of 3.0 and 4.1 lb. per application per area of 100 sq. yd. Injury to the seedlings resulted from the use of the larger amount, whereas satisfactory control without injury to the seedlings followed the use of 3 lb. applied on alternate nights or at successive intervals of 3 nights. Satisfactory control resulted from treatments made every fourth night in some of the trials, provided the covers were thoroughly wetted.

When it became apparent that PDB may act as an eradicant fungicide and may be used successfully to control downy mildew, either by nightly applications or according to a fixed schedule in which the interval between applications is longer, attempts were made to test the effectiveness of applications made at irregular times, as indicated by best judgment. Only infected seedbeds were used in these experiments. Several factors were taken into account in judging when to treat and in approximating the amount to

apply. The severity of the disease and weather conditions were of primary importance in judging when to apply PDB. Some of the treatments were made in the daytime to take advantage of the influence of increased temperature on the rate of vaporization of the crystals. This factor, together with the volume of the seedbed, was taken into account in judging the quantity of fumigant to apply. In all cases the crystals were broadcast upon the thin covers just prior to fitting the heavier covers over the beds. A total of 1364 sq. yd. of seedbed was treated in this series of experiments, with the result that satisfactory control was accomplished in all. However, since the season was not an extremely severe one with respect to incidence of the disease, further experience with the above procedure involving applications at irregular intervals should be obtained before it is generally recommended as applicable to all conditions of downy-mildew incidence.

#### GENERAL CONSIDERATIONS

The concentration of PDB vapor in water that inhibits germination of sporangia of *Peronospora tabacina* (4) has been found to be of the same order of magnitude as that that is fungicidal, as determined by laboratory studies (2) using infected plants maintained in an atmosphere having a constant volume-percentage PDB vapor concentration. All of the measurements of volume-percentage vapor-concentration in the atmosphere of seedbeds, however, as shown in tables 1, 2, and 3 yield a lower figure. The explanation of this apparent discrepancy lies in differences in conditions existing between laboratory and seedbed experiments. In the laboratory, infected seedlings were maintained at a constant temperature and a constant concentration of vapors for periods of 12 hours, so that a condition was reached wherein the PDB content of the air was in approximate equilibrium with that of the plant tissues. Such an equilibrium condition is, no doubt, never approximated in seedbeds. The vapors of PDB, however, appear to be extensively absorbed by the plants, the soil, and the wet covers; the soil, in particular, serves as a reservoir for storage of dissolved PDB. The fumigant reevaporates from the soil when the atmosphere in the bed loses its vapor content through leakage or otherwise.

The persistence of the odor of PDB in the atmosphere of seedbeds until late in the morning after the covers are removed may be regarded as evidence of absorption and reevaporation of PDB vapors by the soil and the seedlings.

The low vapor pressure of PDB at the lower temperatures and its relative insolubility indicate that this fumigant might not be equally effective in all seasons against tobacco downy mildew in seedbeds. While the results of our laboratory experiments (2) show that the pathogen yields to control if two or three applications of 12 hours each are made on successive nights with concentrations of PDB corresponding with saturation at 0° C., it is difficult if not impossible to approach such saturation concentrations at the same temperatures in the seedbeds. In case extended periods prevail during

which the temperatures remain below 7° C., the use of PDB might not control, as has previously been pointed out in this report in experiments involving the influence of temperature.

Manifestly, the employment of any measures during cold weather, which would increase the vaporization of PDB and would minimize the loss of vapors from the bed, would make it more nearly possible to approach concentrations that are effective. In cold weather the use of larger quantities of properly distributed PDB crystals would increase the area of the crystal surface whence vaporization could take place, and, therefore, would tend to build up higher vapor concentrations within the bed. Of course, much of this larger amount of crystals would be found, in the morning, to have remained unvaporized.

Even though the periods of cold weather lasted 3 or 4 days, it might still be possible to control tobacco downy mildew by the use of PDB. This could be accomplished (a) provided the pathogen had been eradicated within the beds prior to the advent of the period of cold weather, or if, by fumigation, the seedlings had been completely protected against infection before the cold weather began, and (b) provided the duration of the cold period was less than the length of the sporangial cycle, *i.e.*, 5 to 7 days. Control might also be accomplished by allowing the heavy covers to remain in place in the morning until the seedbeds have been warmed by the sun to the point where fungicidal concentrations of PDB vapors had been built up. The length of time that covers may be permitted to remain on the beds in the morning must be carefully limited, because experience has shown that concentrations toxic to the seedlings may develop if the covers are not removed after 2 or 3 hours of sunshine.

In spite of these limitations imposed by cold weather, the results of the past 2 seasons have abundantly shown that, under ordinary weather conditions, PDB can be applied as an effective control of tobacco downy mildew. The application of the results of these field studies to seedbed practices indicates that the successful use of PDB as a fumigant requires an appreciation of the interdependence especially (a) of temperature conditions, (b) of tightness of seedbeds as affected by construction of frames and by moisture on the covers, (c) of quantity of the fumigant applied per unit area of seedbed, and (d) of proper distribution of the crystals to be vaporized.

#### SUMMARY

Paradichlorobenzene has given satisfactory control of downy mildew of tobacco in seedbeds, either when applied on successive nights or over longer intervals between successive applications.

This volatile compound may be regarded as an effective fungicide against tobacco downy mildew when employed under the following conditions: (A) The proper amount of paradichlorobenzene is from 1.5 to 3 lb. per application per seedbed area of 100 sq. yd. (B) The crystals should be distributed widely on the top of the loose-texture cloth ordinarily used for seedbed

covers. Sheeting of approximately 60 threads each way per inch should be used as a covering during fumigation. (C) Under seedbed conditions temperatures above 7° C. are necessary for vaporization to proceed sufficiently to maintain effective vapor concentrations. (D) Moisture on the covers is desirable as an aid in retaining effective concentrations of vapor within seedbeds.

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#### NEW STAGES OF SPOROCYBE AZALEAE

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*Sporocybe azaleae* (Peck) Sacc. causes a bud and twig blight of azaleas and rhododendrons. This fungus is imperfectly known but is generally recognized by coremia that appear on the infected flower buds, the leaf and stem buds, leaf scars, old stems, floral parts, and on the fruits.<sup>1</sup>

Peck,<sup>2</sup> in 1873, named this fungus *Periconia azaleae*, but Saccardo,<sup>3</sup> in 1886, transferred the genus to *Sporocybe*; since then it has been known as *S. azaleae*. This Latin binomial refers to the "spore-heads" of the fungus heretofore described. Relying on this description, mycologists have placed the fungus in the taxonomic keys adjacent to *Graphium ulmi* Schw., cause of the Dutch elm disease.

The separation of these 2 genera is based on color of the coremia and spores. Those with light-colored coremiospores are *Graphium*; those with dark-colored ones are *Sporocybe*.

*Graphium ulmi* has 3 spore stages or forms listed; a coremial stage assigned to *Graphium*; a conidial stage assigned to *Cephalosporium* and an ascogenous stage assigned to *Ceratostomella*. *Sporocybe azaleae*, however, has only the coremial stage listed. Since these 2 fungi are taxonomically related, one might expect them to possess similar stages in their life histories.

<sup>1</sup> Description of the disease, pathological anatomy, methods of culturing the fungus, together with inoculations of hosts, have been described in *Phytopath.* 29: 517-529. 1939.

<sup>2</sup> Peck, C. H. *Periconia azaleae*. New York State Museum of Natural History Ann. Rpt. 25: 93. 1873.

<sup>3</sup> Saccardo, P. A. *Sylloge Fungorum* 4: 608, 1886; 10: 692-693, 1892; 11: 643-644, 1895; 12: 744, 1897; 20: 861, 1911.

An investigation was undertaken in 1930 to test this supposition by culturing the fungus.

Monosporous cultures were prepared by isolating coremiospores (Plate I, I) removed from azalea buds and germinated in van Tieghem cells. From these cultures mycelium was implanted on potato-dextrose agar and incubated at 22° C.

#### HYPHAE, MYCELIUM, AND SCLEROTIA

Young hyphae at the margin of a maturing colony, incubated on potato-dextrose agar for 1 week, appeared either gray or nearly hyaline (Fig. 1, F and Plate I, A). The average weekly growth of 6 of these cultures incubated at 22° C. was 4 mm. radially and 2.7 mm. vertically. However, when maturing, they changed from gray to either a citrate drab or to a deep gray-olive, but sporulating hyphae were often a snuff-brown. The cells in vegetative hyphae growing on artificial media averaged  $4 \times 8 \mu$ , but, when growing in host tissues,  $3 \times 5 \mu$ . In a sclerotium some of the cells were rectangular,  $1.7 \times 3 \mu$ , while others emerging were much larger in diameter. One such hypha was  $6 \mu$  in diameter and consisted of cells 54, 72, 18 and  $58 \mu$  in length. Cells in sporulating hyphae that formed conidiophores averaged  $1.8-2.7 \times 4-29 \mu$  (Fig. 1, D).

The cell walls of old hyphae located at the center of mature cultures were either a snuff-brown or a warm-sepia. When located in the substratum and viewed by direct light, they were brownish but, by reflected light, a blue purple undercolor which gave the culture a distinguishing characteristic.

Old hyphae often are constricted at their cross walls; the branching is sparse and forms angles of 80° to 90° (Fig. 1, F; Plate I, H). In cultures the first branching was noted after an incubation of 72 hours, when the germ tubes averaged  $300 \mu$  in length.

Hyphae sometimes entered host cells and formed "skeins" and "knots" which are here considered haustoria. In no case were woody vessels observed to be plugged by these hyphal knots.

On water agar, the hyphae that gave rise to conidiophores remained a light-gray and sparsely septate. They quickly permeated the agar, leaving behind only a sparse surface growth to form cephalospores. On nutrient agar, however, the hyphae were dark-gray or brown, slower to penetrate the substratum, their cells larger, and they formed a definite mycelial mat or subicle, with an abundance of spores. Thus it is to be noted that the nature of the substratum or agar varied the size, shape, color, and amount of hyphae.

Sometimes cultures formed pionnotes, especially when produced through transfer from old cultures that had been incubated for 6 months. This type of culture sometimes "reverted color" to a pale cartridge-buff; but, at maturity, changed to a pale pinkish-orange due to the separation of numerous conidia broken from the sporogenous heads. These conidia budded copiously, giving the appearance of a bacterial contamination.

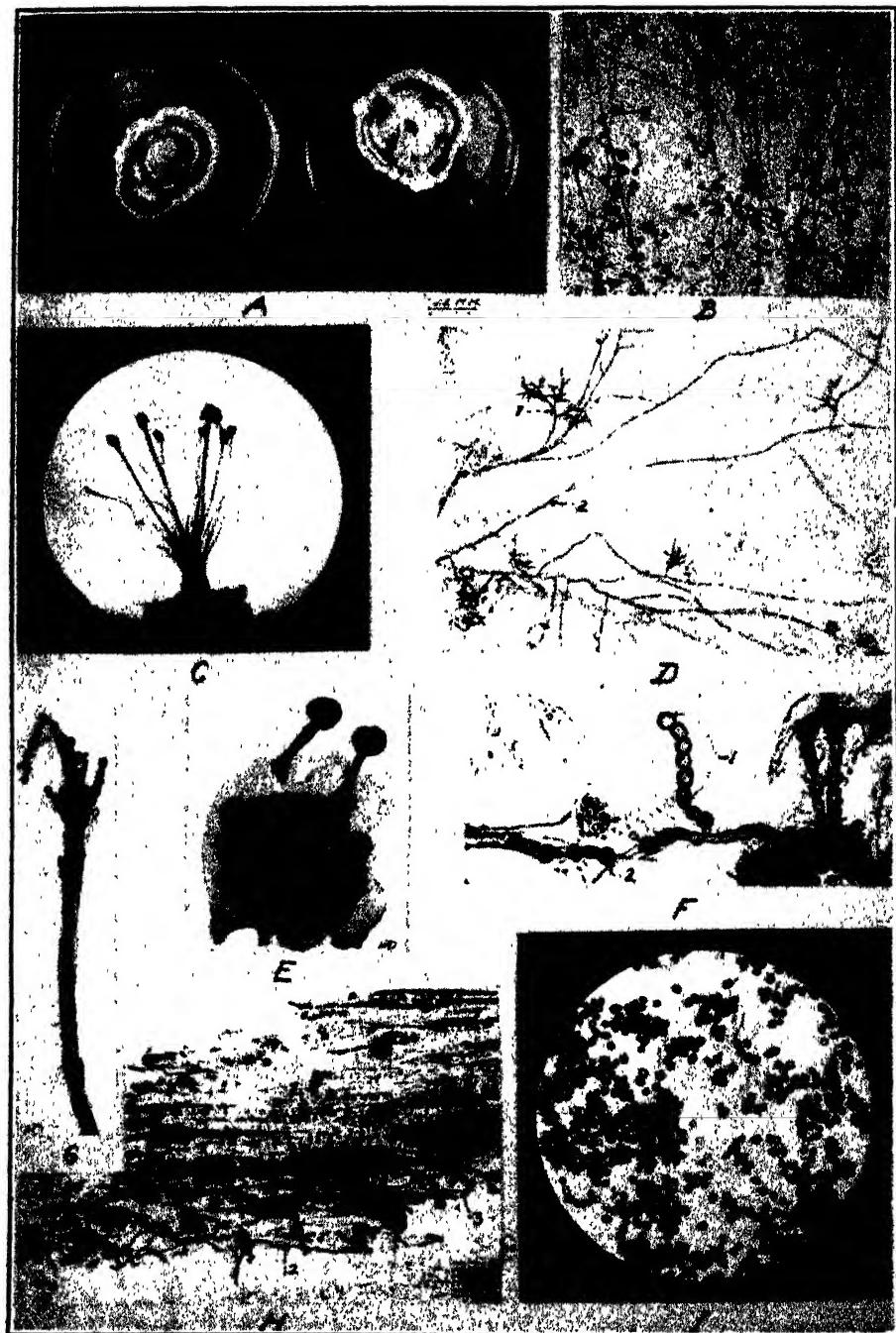


PLATE I. Photographs of cultures, fruiting bodies, spores and hyphae of *Sporocybe esalaeae*.

A. Two immature, monosporous cultures transferred to potato-dextrose agar on February 14; incubated at 22° C. and photographed the following March 10. Dark concentric areas locate the penicillloid form of sporulation.

Transfers from pink cultures, incubated at 28° C., produced a moist, strict growth with pionnotid and penicilloid forms of *Sporocybe*.

Four nonsporulating "albino" strains of this fungus were isolated but were not employed in the experimentation. They, however, were transferred and retained in culture for 2 years without sporulation. They are considered as a spermogonial form.

Sclerotia were formed on the surface by hyphae emerging from substrata such as agar, azalea buds, stems, leaf petioles, and floral parts (Fig. 1, M and Plate I, E). In the presence of sufficient sunlight and air, they generally formed on the surface of the receptacle, uncovered and covered by the agar, but they may form also on other materials adjacent to nutrients and in host tissues. Small sclerotia formed in one culture prepared by spreading coremiospores on cherry-dextrose agar and incubating in van Tieghem cells. After 48 hours, the germ tubes had branched and intertwined, forming sclerotia (stromata) averaging 35  $\mu$  in diameter and 1.8 to 10  $\mu$  thick. They first appeared in cultures as gray flecks with minute white centers, but later became black and papillate, and measured 18 to 65  $\mu$  in diameter. Some of the papillate sclerotia formed matured coremia. Fully matured sclerotia in and on host tissues differed in size, shape, and structure. On old bud scales, they averaged 84  $\mu$  in height and 109  $\mu$  in width.

A large typical sclerotium was composed of layers: epidermal and cortical, and a central core containing most of the viable cells distinguishable by vital staining. The core was generally the source of the coremial stipe (Fig. 1, O).

After overwintered sclerotia had been incubated for 24 hours in a suitable environment, sturdy hyphae emerged through their apices and formed mycelial colonies about 0.5 mm. in diameter. Tests showed that sclerotia remained viable on azalea buds for 2 years and generally produced but 1 coremium annually; however, two coremia have been observed on a single

B. False spore "heads" of the cephalosporic stage, cultured on potato-dextrose agar; 16–20  $\mu$  in diameter; strain D; set July 4 and photographed July 28. 1. Individual spores showing in a "head." 2. "Slime" conceals individual spores, which formed in most of the "heads."

C. A partially dissected coremium showing a synema (stalk) composed of parallel hyphae; a caput (head) of coremiospores; a sclerotium (subiculum) supporting the stipe and the attached bud scale; see also G.

D. Conidiophores of the penicilloid stage bearing chain conidia; a stage following the formation of cephalospores; a culture prepared July 27 and photographed on August 9. A region located in one of the dark concentric rings shown in A. 1. Conidiophore of the penicilloid type bearing conidia in chains. 2. Cells in a vegetative hypha.

E. Coremia and sclerotia on 1933 bud scale tissues of *Azalea nudiflora*; photographed September 6, 1934. Mounted in lactophenol-green.

F. Chlamydospores cultured on potato-dextrose agar in a van Tieghem cell; incubated from April 15 to June 17; average diameter 7.2  $\mu$ . 1. Vertical spore chain. The positive was partially bleached and traced with ink. 2. Chlamydospores as photographed.

G. Portion of a synema (stalk) of a coremium showing its parallel conidiophores (hyphae) and terminal conidia in chains.

H. Vegetative hyphae in an azalea stem; tangential, free-hand section just below an infected bud; stained and mounted in lactophenol-green. 1. Tyloses in cells of the medullary ray. 2–3. Hyphae are both inter- and intracellular.

I. Photomicrograph of coremiospores mounted in lactophenol-green July 29; average diameter of spores 5.4  $\mu$ .

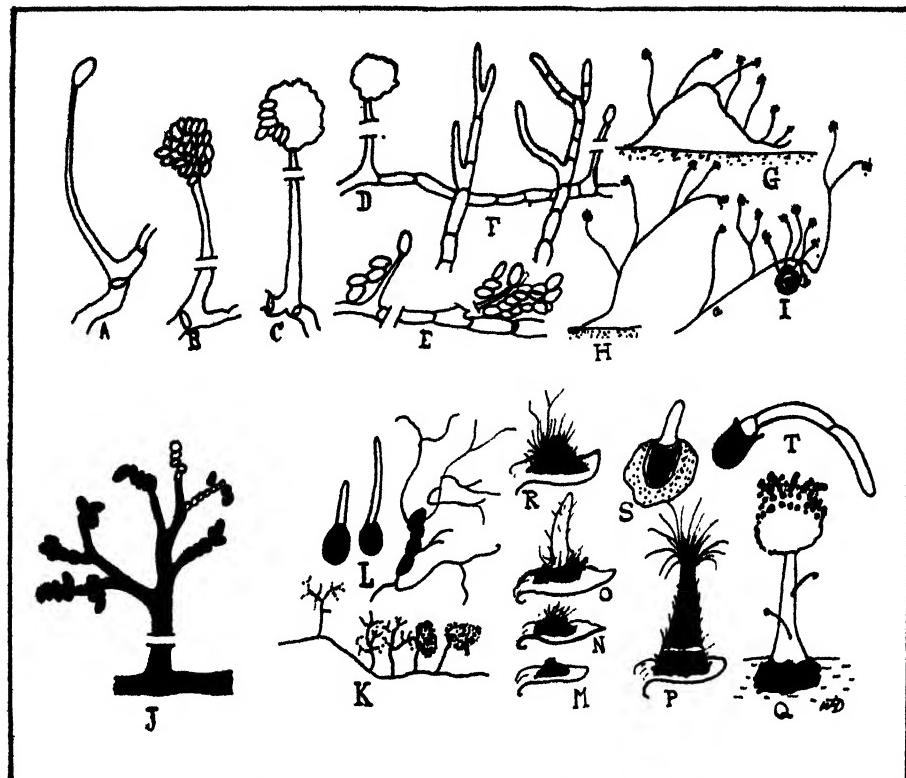


FIG. 1. Tracings of camera-lucida drawings showing the cephalosporic, penicilloid, and coremial stages of *Sporocybe azaleae*. A. A conidiophore with its terminal cell abstricted thereby forming the first conidium of a "false head" or caput. B. Abstricted conidia forming a caput. C. A matured caput with conidia in a "droplet" or false head; conidia  $2-4 \times 3-5 \mu$ . D. Arrangement of conidiophores on a sporulating hypha cultured on cherry agar; conidiophores 63 and 44  $\mu$  long. E. Conidia in two "false heads" separating after drying for 4 months. F. Two hyphae showing the sizes of cells and method of branching after they had been incubated for 56 hours on cherry agar in a van Tieghem cell. At the left, a young actively growing hyaline hypha near the margin of the culture; branches projected at an angle of  $30^\circ$ . At the right, a matured hypha; cells with dark walls and shorter in length; 40, 72, 10, 55, 50, 50, and 50  $\mu$ , respectively; cells in A averaged 3 times this length. G, H, I. Arrangement of conidiophores on sporulating hyphae. Few conidiophores branched as 1, a; basal portion 16  $\mu$  bearing branches or forks 32  $\mu$  long. Whorls are often formed and here conidiophores are more numerous than I, b. Air currents raised part of 8 above the agar. J. A dendritic or penicilloid conidiophore formed in culture after incubated for 30 days on potato-dextrose agar; 66  $\mu$  tall; conidiophores and conidia were a smoky color when matured but nearly hyaline when first formed and averaging 5  $\mu$  in diameter. K. Arrangement of dendritic conidiophores on a sporulating hypha. Distances between the first two conidiophores at the left, 100  $\mu$ ; others averaged 67  $\mu$  apart. L. Conidia of the penicilloid form germinating after incubating for 12 to 48 hours. M-Q. Stages in the formation of a coremium from a sclerotium, during April. M. Overwintering sclerotium on a bud scale. N-O. Emergence of coremial hyphae. P. The caput, forming from the hyaline hyphal tips, while the basal portions are dark colored. Q. A matured coremium with the sclerotium; synema, stalk or stipe; caput, with coremiospores borne both singly and in chains, but mostly in chains. Partially diagrammatical. R. Renewed growth in an old sclerotium which had borne one coremium. S-T. Coremiospores germinating in water after incubating for 12 hours. S. Germ tube emerging from a coremiospore; exosporium surrounded by a slime capsule. T. Coremiospore incubated for 24 hours; slime capsule dissolved.

sclerotium. Thus, the sclerotia are reproductive bodies, which tide the fungus through adverse conditions and form either coremia or mycelium. Although these structures are considered stromata by some, the writer prefers to designate them as sclerotia (Fig. 1, M-P).

#### THE CEPHALOSPORIC STAGE

Cephalospores were observed on sclerotia, coremia, between bud scales, on infected azalea buds, rotting bark, old infected stems, decomposed floral parts, nutrient solutions and media. Cephalospores formed in "false heads" when coremiospores were incubated on water agar for various periods. In some of these cultures, hyphae from germinating coremiospores came in contact with each other, intertwined, and formed a spiral from which phialides (conidiophores) grew singly and vertically. These phialides were neither delimited by cross walls within themselves nor by partitions at their junction with the "parent hyphae"; old ones, however, sometimes bore cross walls. They were often larger in diameter at the base than at the apex and of varying lengths (Fig. 1, A-I, and Plate I, B).

#### PENICILLOID STAGE

Penicillloid conidiophores formed on the nutrient agar (Fig. 1, J-K, and Plate I, D) of each cephalosporic culture that had been incubated for one to three weeks or had been exposed to desiccation. This stage was observed also in each monocoremiospore culture on diseased floral parts, buds, twigs, and fruits. Its incidence in hundreds of monocoremiospore cultures gave evidence that it was a conidial form following the cephalosporic stage. Germinated conidia produced forms identical with those produced by coremiospores.

The conidiophores were formed singly, were dark-brown, and grew vertically from a parent, sporulating, prostrate hypha. They bore an unbranched base or trunk with a spreading, dendritic caput composed of sporogenous branches with the spores attached end to end. Drying conidia changed from a light-brown to sepia, abstricted readily and germinated in tap water.

It is to be noted that each conidiophore with its branches appears to be an independent sporiferous element of a corenium. It originates from a sporulating hypha rather than from a sclerotium and develops singly rather than in a group or synema. These conidia remained intact for a longer period than those in a compact head. Since the conidiophore with its conidia has a slight resemblance to *Penicillium*, it may be known as a penicillloid stage or form of *Sporocybe azaleae*.

#### THE COREMIAL STAGE

Most of the coremia formed on the scales of flower buds but they were also observed on leaf buds, twigs, leaf scars, petioles, flower pedicels, floral parts, capsules, and excised host parts lying on soil. Coremia also de-

veloped on most of the media employed for culturing the fungus, but sterilized, wet filter paper in Petri dishes was the substratum most commonly employed. By using inoculum from a monosporous strain, C-13, coremia formed after incubating the agar cultures at room temperatures for 2 to 6 months. Potato dextrose agar, steamed azalea buds, twigs and leaf petioles were favorable substrata but malt and prunes were unsuitable on account of their rich sugar content.

Most of the coremia developed from the centers of small sclerotial bodies or subicula (Fig. 1, M-Q, and Plate I, C and E). In bud scales, they averaged 109  $\mu$  wide and 84  $\mu$  in thickness. From the center of each sclerotium, a stipe or synema appeared first as a conical papilla of light-gray hyphae emerging at right angles to the surface of the sclerotium (Fig. 1, O). Hyphae at the center of the stipe grew more rapidly than those at the periphery where sporulating hyphae became free. Finally, a head or caput was formed at the apex of the parallel hyphae of which the stipe is composed. The stipe changed from a light-gray to brown while the immature caput remained gray but when both were fully matured, they were a dark-brown or appeared black to the unaided eye. The tips of these hyphae consisted of conidia or conidiophores, mostly borne in chains but sometimes single. Dissection showed these conidiophores may branch similarly to the penicilloid form. The weight and flexibility of the conidal chains caused hyphal tips in the synema to droop so as to form the caput (Fig. 1, P).

The cultures of *Sporocybe azaleae* were especially interesting, since the fungus grew slowly over the surface of the agar or about 4 mm. daily, changed from a light-gray to a dark-olive color and bore rings of sporulation induced by alternate periods of light and darkness. They produced several forms of fruiting bodies that bore spores of the penicilloid form, the hyphae penetrated the agar and, by reflected light, the mycelium was dark purple.

#### THE ASCOGENOUS STAGE

Coremia on infected azalea buds were collected from New Hampshire, New York, Massachusetts, Connecticut, New Jersey, and Virginia. From each of these collections, monosporous cultures were incubated on azalea buds, stems, agars, and many other substrata but did not form perithecia. However, when azalea stems were properly inoculated with two or more strains determined by trial, perithecia formed. From 50 trials, 4 strains were isolated, which, when crossed, produced immature perithecia. Perithecia also were observed on old infected buds that had been incubated 6 months in Coplin breeding jars.

These strains were mated in Petri-dish cultures and stored in damp chambers, but subsequently destroyed by forces uncontrolled by the investigator. The few ascospores, however, that had been obtained by preliminary mating tests were employed in experimentation. The ascospores failed to germinate immediately, but seemed to need an after-ripening period of

several months. Tests of 4 cultures produced a few germinating ascospores that were implanted on potato-dextrose agar. Each of these cultures produced typical cephalospores and penicillloid stages of this fungus. Further investigation, however, is needed to obtain sufficient data.

Measurements in microns of 8 perithecia averaged as follows: Beaks, width 67; length 902.4. Body height 394.8; width 310.8. Spores, 1.8–3.6 × 3.6–9. One perithecium with a broken beak bore a few mature spores; all other perithecia were far less mature. It is to be noted that the length of the beaks was more than twice the height of the body. This morphological character is similar to that of *Ceratostomella ulmi*, which, as previously stated, is closely allied to *Sporocybe azaleae*.

Another attempt is being made to determine strains and produce the ascogenous stage, which, in turn, will produce the conidial forms. Furthermore, the question of ascospore germination must also be solved.

#### DIAGNOSIS

**Cephalosporic form of *Sporocybe azaleae*:** The cephalospores formed on upright conidiophores, or phialides.

**Phialides:** Arising singly and vertically from young, horizontal, prostrate hyphae; hyaline to gray; seldom branched but formed singly; lengths, 12 to 30  $\mu$ , average diameter at the base 2.5; at the apex 1.7  $\mu$ . Function: formation of conidia.

**Cephalospores:** Formed singly as conidia at the apex of phialides only, ellipsoidal and hyaline when forming but oblong-angular and a tint of brown or gray when mature and dry. Sizes: limits of variation, 2.5–4.2 × 5.1–9  $\mu$ . Standard, 3.5 × 5.5  $\mu$  (100 fresh spores measured in culture). Function: dissemination and reproduction.

**Caput:** A "false head" at the apex of the phialide, averaging 20  $\mu$  in diameter and formed by the conidia cohering when abstricted but separating ultimately. Assigned to *Cephalosporium* sp.

**Penicillloid form of *Sporocybe azaleae*:** Observed in cultures following cephalospores and on decaying infected azalea buds, flowers, and branches.

**Conidiophores:** Arising vertically from a horizontal sporulating hypha, average spacing 76  $\mu$  apart, dendritic, base or unbranched trunk, 8 to 15 cells, averaging 60  $\mu$  in height, and 3–6  $\mu$  in diameter.

**Caput:** Composed of 2 to 12 spreading, dichotomous branches or conidiophores each supporting a chain of conidia at the tip.

**Conidia:** Unicellular, catenate or formed in chains of 6 to 10 individuals composing 45  $\mu$  of the tips on each branch of the dendritic conidiophore. Dried conidia are unicellular, ovate to globose; often slightly apiculate, brown; sizes 1.5–5 × 2.8–9  $\mu$ . When matured on agar, 70 per cent were nearly globose and averaged 5  $\mu$  in diameter. When germinated in water on nutrient agar, they form typical *Sporocybe* cultures.

**Coremia Stage of *Sporocybe azaleae*:** Coremia found mostly on the terminal flower buds of azaleas, infected the previous season, or that have remained attached to the host one or more years.

**Synema or Stipe:** It originates from a sclerotium averaging 109 × 84  $\mu$  when in the bud scales. At first, gray, but sepia when mature; 50.4  $\mu$  in diameter and 480  $\mu$  in height (average of 10 matured synemata); seldom, if ever, branched; consisting of cohering parallel hyphae or conidiophores.

**Caput:** Composed of the sporulating free ends of hyphae from the synema; hyphae fail to cohere on account of the spores but bend at their apices; height 106  $\mu$ ; expanded width 188  $\mu$  (average of 10 specimens). At first, gray, then sepia.

**Coremiospores:** Formed in chains, 1 to 9 at the tips of conidiophores in the caput; when dried, the chains are broken so as to form individual spores; seldom, 2-celled; globose-ovate, ellipsoidal and oblong; color varies from gray, when formed, to dark-brown or sepia when mature; sizes, limits of variation 3–10 × 3–17  $\mu$ ; standard, 5.4 × 7.2; deviation 0.638 + (100 fresh spores collected from coremia on azalea buds in the open, May 1935). These spores decreased about 20 per cent in size upon drying; may increase 300 per cent in diameter while incubating during germination; viability, one year.

## SUMMARY

Mycologists have described previously only the coremial stage of *Sporocybe azaleae*, but have placed this fungus in keys adjacent to *Graphium ulmi*, which has cephalosporic, coremial, and ascogenous stages. This investigation was undertaken to determine whether *Sporocybe azaleae* likewise possesses these stages in its life cycle.

Measurements of the hyphae and sclerotia, together with descriptions, are recorded and cultures of the fungus briefly described.

The fungus possesses the following stages: mycelial, chlamydosporic, cephalosporic, penicillloid, coremial, and probably ascogenous. Albino strains and pionnotes also were observed in cultures.

Cephalospores formed in cultures obtained by incubating coremiospores on various substrata.

A dendritic or penicillloid form of sporophore developed on nutrient agar cultures directly following the cephalospores. Conidia that formed on the tips of these dendritic sporophores were similar to those formed on coremia.

Coremia developed from sclerotia that had formed in the flower-bud scales of azaleas. After overwintering, the sclerotia germinated, forming coremia. Morphologically, a coremium comprises an aggregate of penicillloid conidiophores bearing conidia similar in genetic content, size, shape, and color to those of the penicillloid form.

Perithecia were obtained by mating certain strains found among isolates from various localities. The ascospores appeared to require an after-ripening period, the length of which was not determined because the cultures were wantonly destroyed. The perithecia obtained in culture bore long beaks similar to those of *Ceratostomella ulmi* and measurements are recorded in the text. The evidence thus far obtained was insufficient to establish beyond doubt that this is the perfect stage of *S. azaleae*, so the work is being repeated.

From the above, it is to be noted that *S. azaleae* has cephalosporic, coremial, and ascogenous stages similar to those of *Ceratostomella ulmi*. However, its cephalospores, coremiospores, chlamydospores and ascospores were dark colored when mature. In addition, *S. azaleae* possesses sclerotia, chlamydospores, and a penicillloid form.

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VARIATION IN PATHOGENICITY AND CULTURAL CHARACTERISTICS OF THE COTTON-WILT ORGANISM,  
*FUSARIUM VASINFECTUM*<sup>1</sup>

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(Accepted for publication January 10, 1940)

INTRODUCTION

The investigations herein described concern variations of *Fusarium vasinfectum* Atk. as to cultural characteristics and pathogenicity to cotton. A possible explanation also is given of the phenomenon that a variety of cotton may show greater resistance to the Fusarium wilt organism in some localities than in others.

Isolations of the fungus have been made over a period of years from hundreds of diseased plants collected at various localities in South Carolina. A limited number of these isolates were used for experimentation. Selections were made on the basis of differences in cultural characteristics, as well as length of time the isolates had been retained in culture.

VARIATION IN CULTURAL CHARACTERISTICS

Materials and Methods

Thirteen monosporial cultures of *Fusarium vasinfectum* were used. Three of them were derived from fresh isolations from cotton plants that had wilted in the field in 1937. The remainder were cultures originally isolated in 1931 to 1936, but recovered in 1937 from cotton plants infected in the pathogenicity experiments (described later in this paper). Eight to ten monoconidial cultures had been made from each of these isolates. These monosporial cultures showed the same characteristics as the respective mother cultures and only one of each was selected.

The technique employed was essentially that used by Ullstrup<sup>3</sup>. All cultures were grown on nonacidified potato-dextrose agar and kept at 28° C. Subcultures were made by successive transfers of masses of inoculum from one agar slant to the next, at first weekly, and later bi-weekly until 17 such transfers were made. At each transfer, spore suspensions were prepared and 3 to 6 single spores from each culture were transferred to Petri dishes with potato-dextrose agar. By observing these single-spore isolates, variants could be distinguished as they appeared.

Results

Figure 1, A, shows the appearance of 13 isolates at the beginning of the experiment; figure 1, B, the appearance of these isolates after 17 transfers,

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<sup>3</sup> Ullstrup, A. J. Studies on the variability of pathogenicity and cultural characters of *Gibberella saubinetii*. Jour. Agr. Res. [U. S.] 51: 145-162. 1935.

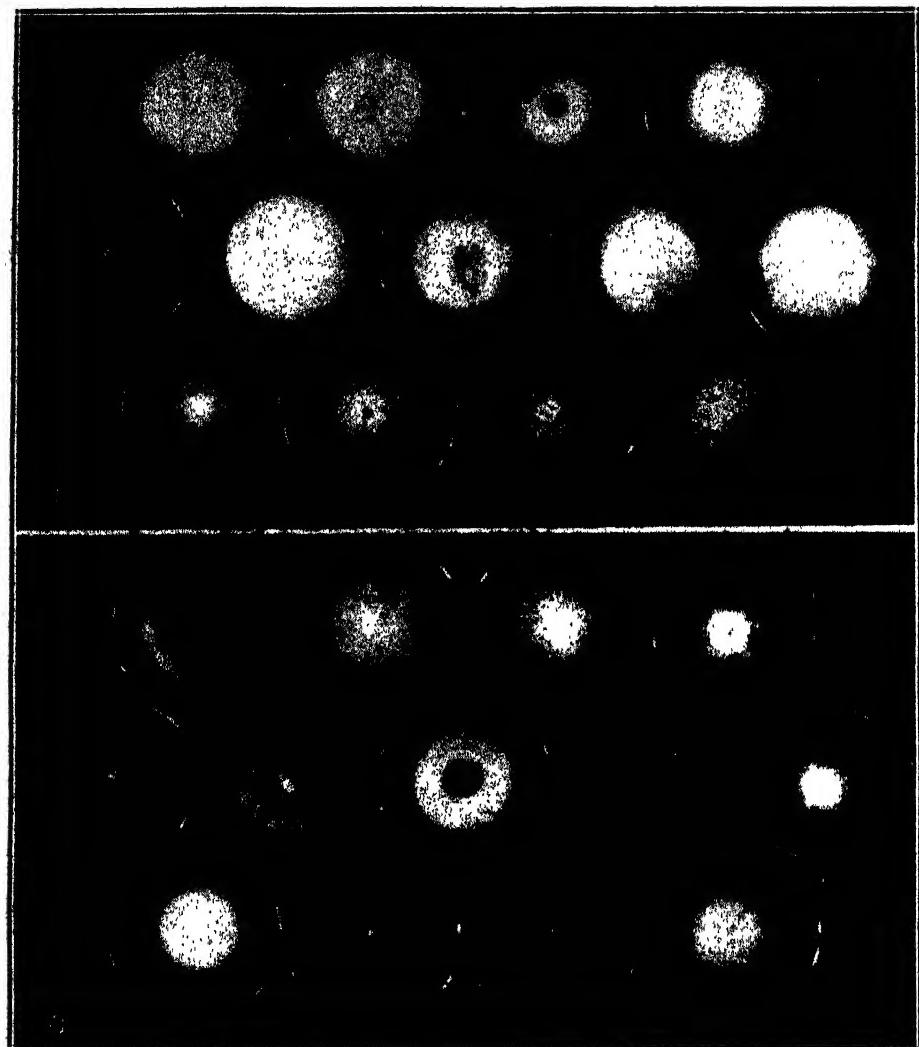


FIG. 1. Variation in the cultural characteristics of *Fusarium vasinfectum*. A. Thirteen different isolates. B. Variants that arose within 17 transfers from isolates represented in A. (Cultures are arranged in the same order in A and B.)

arranged in the same order as in figure 1, A. Eleven of the 13 isolates showed variation in cultural characteristics.

Variations were chiefly in 2 directions; namely, decrease in abundance of aerial mycelium and decrease in the rate of radial growth. No changes occurred in the opposite direction. However, because of the decreased rate of radial growth, some variants appeared to have had denser aerial mycelium than the parent culture.

The occurrence of variation among the respective isolates was irregular, some isolates giving rise to variants at the second transfer but others remaining constant until the 16th or 17th transfer.

Variants generally remained constant in cultural characteristics, but some of them developed secondary variants. Variants had a tendency to dominate the original, even to apparent exclusion of the latter.

An additional experiment was run in which continuous monosporous transfers were made from a single original isolate. The variants appearing in this experiment differed from the original mainly in the rate of growth.

#### VARIATION IN PATHOGENICITY

##### Materials and Methods

In 1937, ten monosporial isolates of *Fusarium vasinfectum* were used to infest soil in which the cotton varieties Farm Relief 2, Semiwilt, and Super Seven were grown. In 1938, 1 recent isolate and 4 cultural variants were added and all were used with the varieties Farm Relief 2 and Dixie Triumph 12. Soil, from a field where cotton had not been grown for at least 5 years, was heavily infested with cultures of the pathogen grown on an oat-wheat mixture.<sup>4</sup> Five 2-gal. pots were used for each isolate and cotton-variety combination. Twenty-five seeds were planted in each pot. A month later, the young plants were thinned to 5 per pot. As soon as a plant exhibited pronounced wilt symptoms, it was removed, examined for internal symptoms, and then a portion of the stem, from just above the ground line, plated on acid potato-dextrose agar. At the conclusion of the experiment, all remaining plants were removed and treated similarly.

##### Results

Data were obtained from 20 to 40 plants per isolate, the average number being 25. The following considerations are concerned primarily with the expression of external symptoms on the susceptible Farm Relief 2 (Fig. 2).

Isolates that had been long kept in culture were less pathogenic than those of recent origin. Isolates 1 and 2 were obtained in 1931, 3 and 10 in 1932, and the remainder in 1936 and 1937.

Four cultural variants were added in 1938. (These are indicated by letters following the number given to the parent isolate.) Variants 5 Z and 5 A were markedly less pathogenic than the parent. On the other hand, variant 12 A and 8 A deviated to a lesser extent from their respective parents.

The relative degree of pathogenicity of the various isolates remained of the same general order in the two successive years. The fungus was re-isolated from diseased plants in the experiments in 1937 and monosporial isolates were made. These isolates were used in the experiments of 1938, excepting No. 12, which was a new isolate.

The single passage of the pathogen through the host plant in 1937 did not significantly modify its relative pathogenicity as expressed in 1938. No

<sup>4</sup> Oat-wheat mixture; 1 bushel of oats and  $\frac{1}{2}$  bushel of wheat were ground together. To this was added  $\frac{1}{2}$  bushel of unground oats and the whole moistened to a crumbly consistency. Transfers of the respective isolates were made to one-quart flasks half-filled with the mixture. The cultures were allowed to grow for 10 days. Inoculum and soil were mixed in the proportions of 1 to 40 by volume.

correlation could be made between the locality from which an isolate originated and its degree of pathogenicity. Isolates 6 and 7 originated from Clemson, in the upper northwest portion of South Carolina; the remainder originated from the lower coastal-plain region of the State.

Positive identity of all slight internal symptoms left correlations between pathogenicity and the presence of internal symptoms open to some question. The same held true on the basis of reisolation of the fungus. One could not be certain in all instances that failure to recover the fungus by plating a portion of the stem could be attributed to complete absence

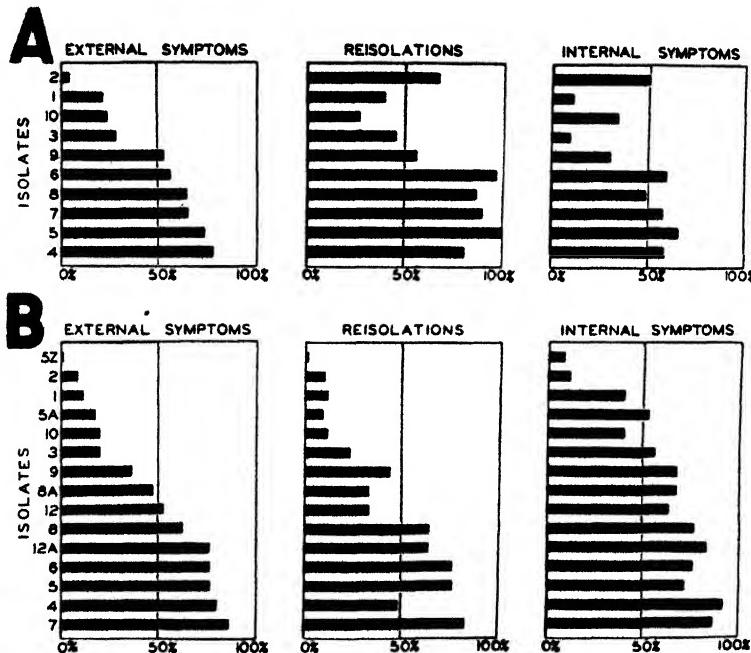


FIG. 2. Relative pathogenicity of different isolates of *Fusarium vasinfectum* to the cotton variety Farm Relief No. 2, expressed on a percentage basis. A. Results obtained in experiments of 1937. B. Results from parallel experiments in 1938 with one recent isolate (12) and three cultural variants (numbers followed by letters).

of the fungus in the plant. Nevertheless, the results obtained are expressed in figure 2. In general, the fungus was recovered, in 1937, from a higher percentage of plants than showed either internal or external symptoms. The experiments of 1938 were started a month earlier than those of 1937, and symptoms of the disease were not expressed until the plants were somewhat older. The lower percentage of recovery of the fungus in 1938 may have been due to greater difficulties in reisolation. The fungus was slower in emerging from the older wood and contaminations were more abundant.

The cotton varieties Dixie Triumph 12, Super Seven, and Semiwilt proved to be much more resistant to the *Fusarium* isolates than did Farm Relief 2. This accords with field observations in general. Based on the

percentage of plants exhibiting external symptoms and from which the pathogen was recovered, the degrees of susceptibility of Dixie Triumph 12, Super Seven, Semiwilt, and Farm Relief 2 were found to be in the order of 2, 3, 4, and 10, respectively. In figure 2 the isolates are arranged in the order of pathogenicity to Farm Relief 2. In general, their pathogenicity to the other 3 varieties followed somewhat the same order, though the percentages of infection and external symptoms in the resistant varieties were so reduced that differences between isolates were less evident.

The number of reisolations made in 1937 from Super Seven and Semiwilt were far in excess of the number of plants exhibiting either external or internal symptoms. For example, isolate 6 was recovered from 100 per cent of the Super Seven plants, but only 28 per cent of the plants exhibited external symptoms and 32 per cent internal symptoms.

A spore suspension was used as inoculum in a parallel experiment in 1938, mixing about 5 billion spores into the soil of each pot. The spores were washed from cultures grown on the oat-wheat mixture. Five isolates (Nos. 5, 6, 8, 9, and 5 Z) of the pathogen were used with the variety Farm Relief 2. In general, the relative pathogenicity of the isolates followed the same order as in the experiments previously described. However, the percentages of infected plants, as well as of plants exhibiting external symptoms, were considerably lower.

#### DISCUSSION

Based on the expression of external symptoms by the cotton variety Farm Relief 2, the isolates of *Fusarium vasinfectum* presented a wide range in pathogenicity. It has been reported for certain other *Fusaria*<sup>5,6</sup> that isolates characterized by a combination of abundant aerial mycelium and rapid radial growth are highly pathogenic. This held true for the isolates 5, 6, 8, and 12. On the other hand, isolates 4 and 7 were relatively high in pathogenicity but exhibited an appressed type of growth on potato-dextrose agar. Isolates 2, 1, 10, and 3, with the appressed cultural character, were lowest in pathogenicity. These phenomena are in accordance with studies of other *Fusaria* and lead to the conclusion that a cultural variant may or may not be less pathogenic than the isolate from which it arose.

It was observed that fresh isolations of *Fusarium vasinfectum*, obtained from diseased plants in the field, are generally characterized by abundant aerial mycelium and rapid growth in culture. In all probability, the very weakly pathogenic isolates 2, 1, 10, and 3 with the appressed cultural character represent variants that had arisen during their lengthy period in culture.

Pathogenicity and cultural characteristics of the isolates were not affected to any extent by a single passage through the host. This is especially

<sup>5</sup> See footnote 3.

<sup>6</sup> Harvey, C. C. Studies in the genus *Fusarium*. VII. On the different degrees of parasitic activity shown by various strains of *Fusarium fructigenum*. Ann. Bot. 43: 245-259. 1929.

interesting with respect to the weakly pathogenic isolates that had long been in culture.

The cotton varieties Dixie Triumph 12, Semiwilt, and Super Seven proved to be considerably more resistant to all the isolates than did Farm Relief 2. Reisolation of the fungus from the resistant varieties was far in excess of the number of plants exhibiting external symptoms. This would indicate that resistance was not involved in the infection process.

Whether or not the development of cultural variants differing in pathogenicity answers the problem of why a variety of cotton seems to be more resistant in some localities than in others may be open to question. Edaphic factors may be of significance. Nevertheless, since this fungus grows saprophytically in the soil, it is possible that variants differing in pathogenicity may occur in the field. Orton<sup>7</sup> obtained "dissociation" of *Fusarium vasinfectum* in sterilized soil.

#### SUMMARY

Evidence is given that variants of *Fusarium vasinfectum* do arise in culture. A variant tends to have less aerial mycelium and to exhibit a slower rate of growth than the parent. None was found that reverted to the parent type during 17 successive transfers. Moreover, a variant appeared to dominate the parent type, even to the exclusion of the latter. A variant may give rise to secondary variants.

Isolates showed a wide range in pathogenicity. Cultures that exhibited abundant aerial mycelium and grew rapidly were among the highly pathogenic group, but variation from this cultural type may or may not be paralleled by decrease in pathogenicity.

Isolates that had long been retained in culture were weakly pathogenic. Their cultural characteristics indicated that they were variants that had arisen in culture.

A single passage of an isolate through the host did not modify its pathogenicity.

The high percentage of reisolation from resistant cotton varieties suggests that the mechanism of resistance is not involved in the process of infection.

It is suggested that variants of this fungus, which differ in pathogenicity, may occur in the field.

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<sup>7</sup> Orton, C. R. The dissociation of *Fusarium* in soil. Bull. Terr. Bot. Club 62: 413-418. 1935.

# THE PRACTICABILITY OF DETECTING DUTCH ELM DISEASE BY TRUNK SAMPLING

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(Accepted for publication January 30, 1940)

The purpose of this study was to devise a practicable method for diagnosing Dutch elm disease by sampling accessible portions of the trunks of suspected elms, and to determine some applications of this method in the control of the disease.

There are limitations to the technique of finding all elms infected with *Ceratostomella ulmi* (Schwarz) Buisman, by culturing twig samples from trees expressing foliar symptoms commonly associated with the Dutch elm disease. True and Slowata<sup>1</sup> found that from 0.007 to 2.3 per cent of such elms in plots sampled in 1936 and 1937 were actually infected with *C. ulmi* and, conversely, that 50 per cent of the trees found infected did not show external symptoms. In addition, collecting twig samples from elms is expensive, since approximately one-half of them are so tall that satisfactory samples cannot be obtained without climbing the trees. Also an accurate inspection for external symptoms is impracticable in densely wooded areas. Furthermore, any scouting method based on the presence of foliar symptoms is handicapped by the shortness of the period during which it can be employed.

The occurrence of both the fungus and the vascular discoloration associated with it in the trunks of infected trees has been mentioned by earlier writers<sup>2, 3</sup> and extensively demonstrated by Smith.<sup>4</sup> Therefore, the principal problem in the present study was to determine whether specimens suitable for laboratory diagnosis could be collected from the trunks of elms suspected of having the disease and whether such a procedure would be a practicable method for finding infected elms, particularly during the dormant season. In the course of the study suitable tools were devised, methods for trunk sampling were developed, the effectiveness of the method for finding diseased trees was tested in the field, and the effects of the trunk-sampling operation upon the trees were determined.

## MATERIALS AND METHODS

An arch-type leather punch, extracting a sample  $\frac{3}{4}$  in. in diameter, was used throughout the early part of the work. Later, an inexpensive leather punch having a bore  $\frac{3}{8}$  in. in diameter was found satisfactory. The

<sup>1</sup> True, R. P., and S. S. Slowata. Scouting and sampling elms wth symptoms comonly associated with the Dutch elm disease as an aid in eradicating *Ceratostomella ulmi*. *Phytopath.* 29: 529-537. 1939.

<sup>2</sup> May, C., O. N. Liming, and Thelma Alexander. The Dutch elm disease in Ohio. (Abstract) *Phytopath.* 21: 125. 1931.

<sup>3</sup> Schwarz, Marie B. Das Zweigsterben der Ulmen, Traueweiden, und Pfirsichbäume. Utrecht. A. Oosthoek. pp. 7-32. 1922. Part relating to elm translated by L. K. Kelsey as "The twig wilt and vascular disease of the elm." Bartlett Research Lab. Bull. 1: 5-25. 1928.

<sup>4</sup> Unpublished report. A. L. Smith, formerly Field Assistant, Division of Forest Pathology, Bureau of Plant Industry.

punch was driven into the tree with a 1-pound composition rubber mallet, given a downward thrust to snap off the wood core inside it, and then removed from the tree (Fig. 1, A). The wood core was ejected from the punch with a wooden plunger set in the handle of the mallet.

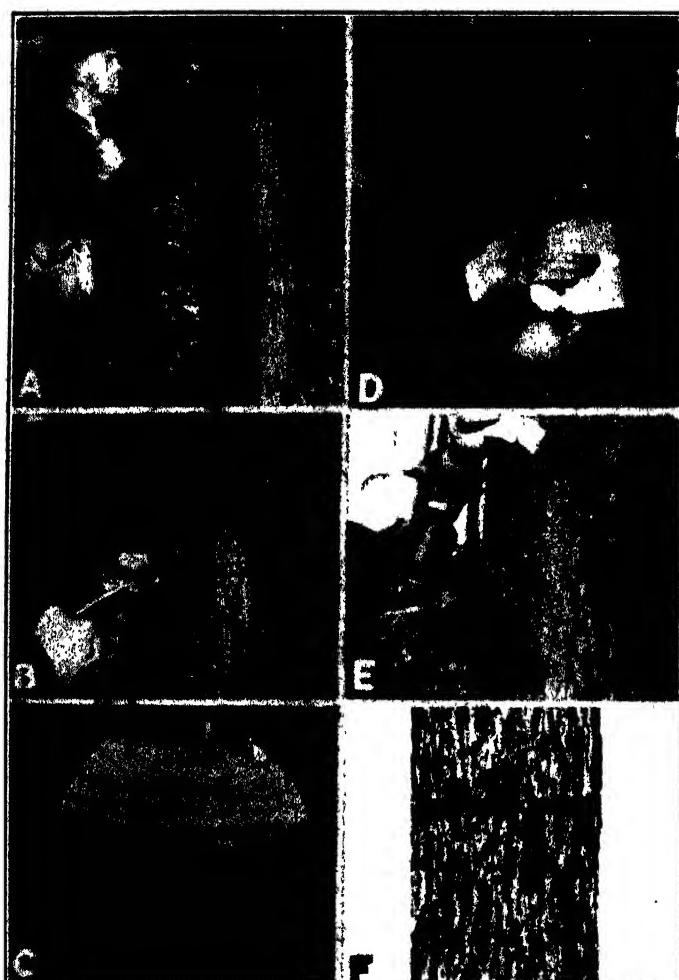


FIG. 1. Steps in trunk sampling and a healed injury. A. Removing samples. B. Slicing sample previous to inspection for vascular discoloration. C. Typical vascular discoloration in sliced trunk sample. D. Collecting samples for culturing. E. Painting the wounds. F. Healed wound 1 growing season after sampling.

The samples were taken on the circumference of the tree at a height convenient to the operator, usually the shoulder line. The spacing of the sampling points was based on unpublished studies by Smith<sup>6</sup> of the distribution of vascular discoloration in trunks of infected elms. He examined cross sections of 284 elms showing Dutch elm disease and found that 242 (85 per cent) contained some discoloration at breast height. By examining

<sup>6</sup> See footnote 4.

incisions spaced at 3-, 6- and 12-in. intervals on the circumference of these 242 trees, he detected vascular discoloration at one or more points in 95.5, 93, and 78 per cent of the trees, respectively. Since incisions at 3-in. intervals were only 2.5 per cent more effective than those at 6-in. intervals, samples taken by the author during the dormant season were spaced approximately 6 inches apart.

Because of variation in growth increment and thickness of bark, the sampling depth was based on the number of annual rings penetrated rather than on a linear measure. The internal symptoms of the disease often occur in the wood of annual rings infected prior to the current year; therefore, the samples included wood from no less than 2, but usually no more than 5, annual rings.

All the samples were sliced diagonally through the center and across the grain (Fig. 1, C). Those  $\frac{3}{4}$  in. in diameter were sliced with a pocket knife; those  $\frac{1}{2}$  in. in diameter were sliced with especially adapted pruning snips (Fig. 1, B).

When discoloration was detected in a sliced sample, the subsequent discolored samples from the trees were retained without slicing. In most cases only 1 or 2 plugs from each tree contained discoloration and, therefore, 3 additional samples were taken in a vertical line with the hole from which the sliced discolored plug was removed (Fig. 1, D). After the outer bark had been removed from these samples, they were placed in a glassine bag, properly labeled, and later cultured on potato-sucrose agar. The punch was sterilized with alcohol if discoloration was found.

A pump-type oiling can was converted into a suitable instrument for applying wound dressing to the  $\frac{1}{2}$ -in. in diameter holes from which samples had been taken. The position of the exit holes on the nipple and the pumping action spread the paint evenly over the inside surface of the wound (Fig. 1, E).

#### EFFECTIVENESS OF TRUNK SAMPLING

##### A. Sampling During the Dormant Season

During the dormant season of 1936-1937, samples were taken at 6-in. intervals from the trunks of 6,031 elms to study the effectiveness of this method for finding infections of *Ceratostomella ulmi*. The elms sampled had been inspected for foliar symptoms during the previous growing season, and 114 of them being found diseased, were eradicated before the trunk sampling was done. Elms smaller than approximately 3 in. in diameter, breast high, were not trunk-sampled because twig samples could be collected in less time. The results of this study are given in table 1.

Forty-five new *Ceratostomella ulmi* infections were discovered within the plots. These increased the total number of cases for the period of the study from 114 to 159. Extensive patterns of vascular discoloration in the 45 trees indicated that they had been diseased during at least the final scouting period preceding trunk sampling. There were occasional

TABLE 1.—Additional infections of *Ceratostomella ulmi* found by trunk sampling dormant elms at 6-inch intervals in areas from which reedy-  
nized elms had been removed during the previous summer.

Plot name and location	Date of sampling trunks	Trees trunk-sampled			Infected trees eradicated during previous summer*	Number	Percentage found by trunk sampling	Total infected trees found
		Number	Number cultured	Number yielding <i>C. ulmi</i>				
Convent, Morris Co., N. J.	October 1936	1,036	123	23	49	72	32.0	
Morris Co., N. J. (Assorted plots)	January–March 1937	1,183	106	1	0	1	100.0	
East Orange Water Reservation, Essex Co., N. J.	January 1937	294	16	0	1	1	0	
Milltown, Somerset Co., N. J.	January 1937	251	31	13	47	60	21.7	
Raritan, Somerset Co., N. J.	January 1937	569	50	3	5	8	37.5	
Westchester Ave., Westchester Co., N. Y.	January 1937	309	24	0	2	2	0	
Armonk, Westchester Co., N. Y.	January 1937	350	16	0	0	0	.....	
Congers, Rockland Co., N. Y.	March 1937	1,373	59	4	6	10	40.0	
Nanuet, Rockland Co., N. Y.	March 1937	666	14	1	4	5	20.0	
Total		6,031	439	45	114	159	28.3	

\* From records of Dutch Elm Disease Eradication Office, Bureau of Entomology and Plant Quarantine.

galleries of *Scolytus multistriatus* Marsh. and *Hylurgopinus rufipes* Eich. in many trees. Adults of the former had recently emerged from heavily diseased portions of 3 trees.<sup>6</sup> Probably those 3 and possibly others among the 45 had been instrumental in further spread of the fungus.

That trunk sampling is not a method for finding all *Ceratostomella ulmi* infections was demonstrated by discovery of 4 diseased trees among 80 elms removed from the Milltown plot after the trunk sampling was completed. However, vascular discoloration in those trees was not extensive.

The three foliar inspections of the elms in the Convent and Milltown plots during the summer preceding trunk sampling required 72 man days.<sup>7</sup> Trunk sampling the plots consumed 28 man days.

These results suggest several ways in which trunk sampling during the dormant season could be employed in the control of Dutch elm disease: (1) to supplement summer scouting, particularly in areas in which an unusually large number of infected trees had been found by summer scouting; (2) to appraise the effectiveness of summer scouting by sampling plots selected at random; (3) to supplant summer scouting in wild or semi-wild areas adjacent to concentrations of valuable elms.

#### B. Sampling While Trees Are in Foliage

To determine the efficiency of trunk sampling as a method for collecting samples from trees expressing symptoms of Dutch elm disease, a study was conducted during the period June 16 to July 7, 1937, on 66 known diseased trees selected at random in Morris County, New Jersey. They were sampled at 3-in. intervals within 8 to 16 days after discovery by field scouts. *Ceratostomella ulmi* was isolated from trunk samples from 64 of the 66 trees sampled. Sampling at 6-in. intervals would have found 61, (92.4 per cent) of the trees.

Samples from 54 of the 66 trees (82 per cent) contained vascular discoloration in the 1936 annual ring. The circumferential distribution of this discoloration was such that approximately 75 per cent of the 66 trees could have been found by trunk sampling at 6-in. intervals during the preceding dormant period. However, in another group of trees only 31 per cent of 1,300 diseased trees removed later in the same season (July 7 to September 30, 1937) contained discoloration in the 1936 annual ring.

A further test of trunk sampling during the growing season was conducted during the period July 12 to August 25, 1937. Samples were taken in the usual manner at 6-inch intervals from approximately 5,500 elms not known to be diseased. They adjoined approximately 800 diseased trees discovered about 2 weeks earlier by foliar scouting and scattered throughout the known infected area in New Jersey. It is assumed that they were inspected for foliar symptoms at the time samples were taken

<sup>6</sup> Trees were examined for insect infestation by W. D. Buchanan, Division of Forest Insects, Bureau of Entomology and Plant Quarantine.

<sup>7</sup> Data supplied by Dutch Elm Disease Eradication Office, Bureau of Entomology and Plant Quarantine.

from the infected trees that they adjoined. Evidently they did not express external symptoms at that time, since no twig samples had been collected. *Ceratostomella ulmi* was isolated from trunk samples from 38 (0.7 per cent) of the trees.

Other studies yet to be published, indicate that as high as 20 per cent of the diseased trees found by summer scouting during 1937 did not contain sufficient trunk discoloration 1 to 2 weeks after symptoms were first observed to insure detection by trunk sampling at 6-inch intervals.

The results of the several studies of trunk sampling during the foliar season suggest two uses for this method in the control of Dutch elm disease: (1) To define the probable extent of vascular discoloration in more valuable infected elms to determine whether the infected parts could be excised; and (2) to speed summer scouting by collecting trunk samples from symptom trees that would otherwise require climbing.

#### Some Effects on the Elms

Measurements were taken of healing occurring after one growing season on wounds  $\frac{1}{4}$  in. in diameter made by trunk sampling at 6-in. intervals. The 1,425 wounds were distributed on 469 trees in 19 plots, each containing 16 acres, in Morris County, New Jersey. Healing of all wounds, based on percentage of area calloused, averaged 71 per cent, 29 per cent were completely healed, and 75 per cent were closed 50 per cent or more.

Punches  $\frac{1}{2}$  in. in diameter are recommended, but extensive data are not available on the healing of holes of this size. Alternate  $\frac{1}{2}$ - and  $\frac{3}{4}$ -in. samples, however, were taken May 14 to 17, 1937, from 10 trees at approximately 3-in. intervals on the circumference. Measurements of healing were made the following November. The percentages of the wounds made with the  $\frac{1}{2}$ - and  $\frac{3}{4}$ -in. punches that were completely healed were 52 and 21, respectively.

One year after the trees in the Morris County plots were trunk-sampled a study was made of the effect of sampling on growth increment. The average width (2.16 mm.  $\pm$  .11) of the 1937 annual ring formed on sampled trees the year following sampling was comparable to the width (2.23 mm.  $\pm$  .10) of the 1937 annual ring on unsampled check trees. Evidently the trunk-sampling operation had little effect on the increment of growth during the following year.

The relation between growth increment in 1936 and healing of wounds after sampling in 1937 was determined by comparing the width of the 1936 annual ring with the percentage healing of the wounds. On trees of average growth the wounds were approximately 84 per cent closed. The percentages of healing of wounds on trees below and above average radial growth were 50.7 and 94.0, respectively. One would, therefore, expect most wounds made with the smaller ( $\frac{1}{2}$ -in.) punch on trees of average growth increment to heal in one growing season (Fig. 1, F).

Wood samples from trees previously trunk-sampled were plated to deter-

mine if fungi commonly associated with decay in living trees were present. None was found.<sup>8</sup>

#### SUMMARY

Satisfactory tools and methods have been developed for removing samples  $\frac{1}{2}$  in. in diameter and suitable for culturing from trunks of elms suspected of having Dutch elm disease.

During the dormant season 1936-37 this sampling method was applied to 6,031 elms considered disease-free when last inspected for foliar symptoms in the preceding summer. The 45 new infections discovered constituted 28.3 per cent of all infections found in the plots by scouting for foliar symptoms and by trunk sampling.

Dormant-season trunk sampling was less expensive than intensive summer scouting.

Several studies of trunk sampling during the growing season indicated that approximately 80 to 92 per cent of infected elms showing foliar symptoms could be found by trunk sampling about two weeks after symptoms were observed. Those with vascular discoloration insufficient for detection were usually not severely affected by the disease.

Apparently trunk sampling during the dormant season at 6-inch intervals did not injure the trees, since (a) the growth increment of sampled trees during the year following sampling was not retarded; (b) none of the commonly recognized fungi associated with decay of living trees was isolated from platings of wood adjoining healed and unhealed trunk-sample wounds.

Five ways in which trunk sampling might be used in control of Dutch elm diseases have been enumerated.

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#### FUSARIUM LEAF SPOT OF SANSEVIERIA<sup>1</sup>

LEON K. JONES

(Accepted for publication January 15, 1940)

A leaf-spot disease has been observed on *Sansevieria zeylanica* Willd., and *S. zeylanica* var. *laurentii* Hort. in many of the glasshouses in the State of Washington. Often every leaf on a plant may show roundish, somewhat sunken, reddish-brown lesions,  $\frac{1}{2}$  to 1 cm. in diameter, with yellowish borders (Fig. 1). The spots may be limited to one side of the leaf and develop a raised, corky surface. In many cases, the spots extend through the leaf, and

<sup>8</sup> Identification of most of the fungi isolated was made by L. M. Fenner, formerly Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry. Transfers of unidentified fungi isolated from the samples were examined by Ross W. Davidson, Associate Mycologist, Division of Forest Pathology, Bureau of Plant Industry.

<sup>1</sup> Published as Scientific Paper No. 429, College of Agriculture and Agricultural Experiment Station, State College of Washington.

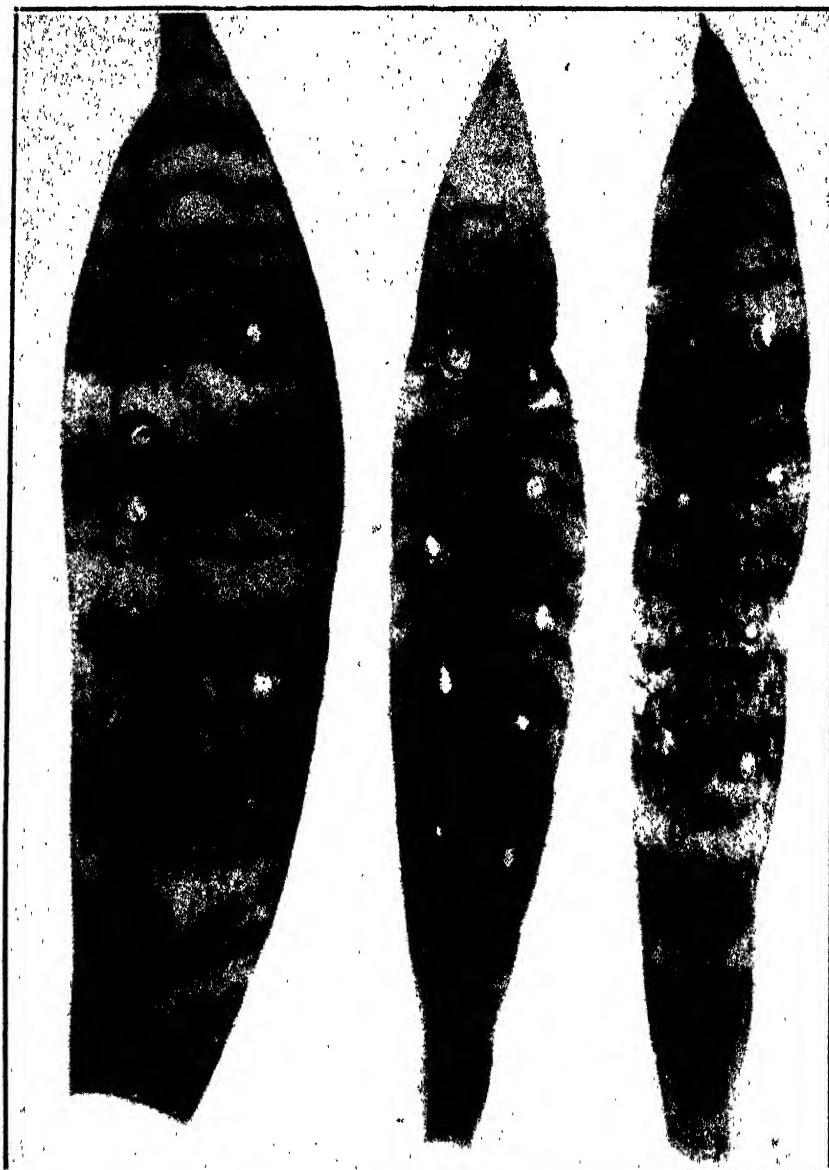


FIG. 1. *Fusarium* leaf spot of *Sansevieria* produced by inoculation in needle punctures with *Fusarium moniliforme*. Center leaf shows girdling by the organism near the tip.

the center dries and falls out. Lesions may sometimes coalesce, encircle the leaf, and cause the death of the distal portion. A similar trouble caused by *Fusarium moniliforme* Sheldon was reported by Kotthoff<sup>2</sup> as common on *Sansevieria* in Germany.

Isolations on potato-dextrose agar were made from 32 spots on 26 affected

<sup>2</sup> Kotthoff, P. Neue Topfpflanzenkrankheiten. Kranke Pflanze 14: 28-30. 1937.

leaves, and 20 of the isolates proved to be a species of *Fusarium*. Bacteria were isolated from 5 spots and another species of *Fusarium* from 2 spots. Inoculations with the 3 organisms showed that the predominant isolate was the only one capable of producing the disease (Table 1).

TABLE 1.—*Results of inoculations to determine the causal agent of Sansevieria leaf spot*

Inoculum	Source of organism	Inoculations			
		Inoc. in injuries	Number infections	Not injured	Number infections
<i>Fusarium moniliforme</i> Sheldon	<i>Sansevieria</i>	87	53	10	6
<i>Fusarium</i> sp.	"	16	0	10	0
<i>Fusarium</i> sp.	Beet	64	0	10	0
Bacterial culture	<i>Sansevieria</i>	26	0	10	0
<i>Fusarium martii</i> App. and Wr. var. <i>pisi</i>	Pea	10	0	10	0
<i>Fusarium conglutinans</i> Woll. var. <i>callistephi</i>					
Beach	Aster	32	0	....	....
<i>Botrytis allii</i> Munn	Onion	42	12	....	....
<i>Penicillium expansum</i> Lk.	Apple	16	0	....	....
<i>Penicillium gladioli</i> L. McC. and Thom	Gladiolus	16	16	....	....
<i>Fusarium</i> sp.	Iris	16	0	5	0
Sprayed with water		5	0		

It was considered that possibly other fungi might be capable of growing in the succulent leaf tissue of *Sansevieria* and, accordingly, a number of common species were used as inoculum (Table 1). The results of these inoculations show that *Botrytis allii* and *Penicillium gladioli* were capable of producing lesions  $\frac{1}{2}$  to 1 cm. in diameter following inoculation into needle-punctured areas. *B. allii* and *P. gladioli* produced circular, water-soaked sunken lesions, those of the former being greenish-brown, the latter greyish-green. The lesions caused by *B. allii* were dried and inactive 14 days after removal from the moist chamber, but the *P. gladioli* lesions were still enlarging after 14 days, and in one case continued and girdled the leaf near the point of inoculation.

The *Fusarium* sp., predominately isolated from *Sansevieria* leaf spot and proved to be the causal agent by inoculation and reisolation, has been determined to be *Fusarium moniliforme* Sheldon.<sup>3</sup>

Infection of *Sansevieria* in injuries made by pricking the leaf with a needle was readily obtained with *F. moniliforme*. Also a number of lesions appeared in uninjured tissue at the base of the leaves where a moisture-holding cup is often formed (Fig. 2). The lesions at the base of the leaves often girdled the leaves and caused the upper portion to die.

It has been recommended that a 4-6-50 Burgundy mixture be used in repeated treatments for the control of this disease. Tests with 4-6-50 Burgundy mixture plus  $\frac{1}{2}$  per cent Penetrol proved to be satisfactory as regards

<sup>3</sup> Acknowledgment is due Dr. C. D. Sherbakoff for verification of determination.

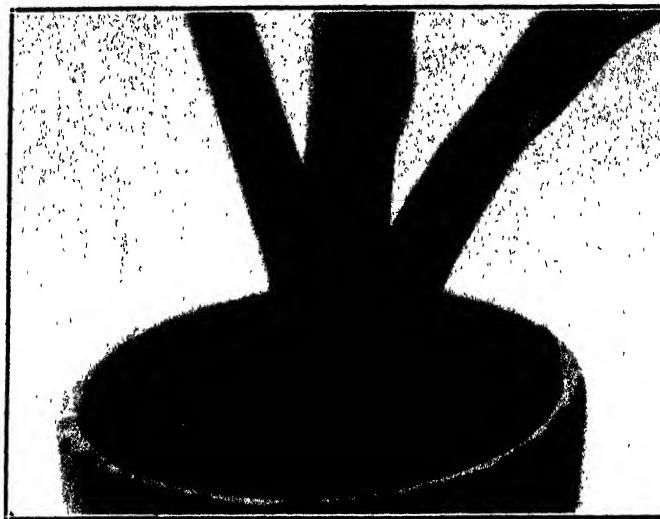


FIG. 2. Infection of *Sansevieria* leaf near the base by *Fusarium moniliforme* following inoculation of uninjured leaves.

coverage and lack of host injury. Sanitary practices in destroying diseased leaves and care in watering so that spores are not washed about on wet leaves should aid in reducing infection.

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#### A PINK STAIN OF WOOD CAUSED BY A SPECIES OF GEOTRICHUM

MAE SPRADLING CHIDESTER  
(Accepted for publication February 16, 1940)

#### INTRODUCTION

A jasper pink<sup>1</sup> or light jasper red stain first attracted my attention when some southern yellow pine lumber was received from New Orleans, Louisiana. This lumber was infected with *Fomes pini* (Thore) Lloyd and the stained wood bordered the decayed portion. The material was believed by the consignor to be so-called red heart, a name commonly used for the incipient stage of *F. pini* rot. However, the color was distinctly different from that of red heart. Moreover, it not only bordered the *F. pini* decayed heartwood but extended out from the heartwood into the sapwood. Hubert<sup>2</sup> described a somewhat similar stain occurring in box elder trees, but no re-

<sup>1</sup> Ridgway, Robert. Color standards and color nomenclature. 43 pp., illus. (Washington.) 1912.

<sup>2</sup> Hubert, E. E. The red stain in the wood of box elder. Jour. Agr. Res. [U.S.] 26: 447-457. 1923.

port of such a stain in pine could be found. Hedgecock<sup>3</sup> reported *Penicillium aureum* Corda and two other species of *Penicillium* as capable of staining pine wood orange red to crimson red, and *Fusarium roseum* Link as the fungus causing pink, red, or violet blotches in pine lumber. Scheffer and Lindgren<sup>4</sup> reported *Fusarium moniliforme* Sheld. as the cause of pink patches in the sapwood of southern yellow pine. Since apparently similarly stained pine had not been described, it was considered desirable to identify the causal agent.

#### CAUSAL FUNGUS

A mold was isolated from the pink-stained specimens. When grown at room temperature (about 25° C.) on malt medium<sup>5</sup> the fungus becomes mealy in appearance in a few days, because of numerous clumps of spores that vary *en masse* from an ivory yellow to a baryta yellow. The malt medium, on which the fungus grows rapidly, soon becomes pinkish. The under side of cultures becomes dark-specked, due to certain portions of the mycelium turning dark brown (Pl. II, A). Some of the mycelium turns a tyrian blue.

The conidia are borne in chains formed by the divisions of the branches of the much and irregularly branched conidiophores (Pl. II, B). The mature spores are hyaline, short cylindrical (Pl. II, C), extreme range 2  $\mu$  to 3.6  $\mu$  by 2.7  $\mu$  to 4.1  $\mu$  and sextile range 2.7  $\mu$  to 3.4  $\mu$  by 3  $\mu$  to 3.7  $\mu$ . The hyphae vary considerably in size, being from 2  $\mu$  to 13  $\mu$  in diameter.

The fungus was referred to the genus *Geotrichum* by Diehl.<sup>6</sup> From a preliminary comparison with descriptions of species of this genus it seems to be a new species, but because of the confusion in the mycological literature concerned with *Geotrichum* and related genera no further attempt was made to classify the fungus or describe it as new.

The author has isolated the same fungus from red-stained cypress heartwood lumber, and Davidson<sup>7</sup> has obtained it from pink-stained heartwood of a decaying oak log.

#### THE STAINING ABILITY OF GEOTRICHUM SP.

The staining ability of *Geotrichum* sp. was tested on the heartwood and sapwood of 8 different species of wood. Cultures originating from a single spore<sup>8</sup> of *Geotrichum* sp. isolated from southern yellow pine heartwood were used to inoculate 10 1  $\times$   $\frac{1}{2}$   $\times$  10 in. sticks, 5 of which were sapwood and 5 heartwood, of each of the following species of wood: silver fir (*Abies amabilis*),

<sup>3</sup> Hedgecock, G. G. Studies upon some chromogenic fungi which discolor wood. Mo. Bot. Gard. 17th Ann. Rpt. (1906): 59-114. 1906.

<sup>4</sup> Scheffer, T. C., and R. M. Lindgren. Some minor stains of southern pine and hard-wood lumber and logs. Jour. Agr. Res. [U.S.] 45: 238-237. 1932.

<sup>5</sup> Trommer's plain malt extract ..... 25 gm.

Bacto-agar ..... 15 gm.

Distilled water ..... 1000 cc.

<sup>6</sup> Diehl, W. W., Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>7</sup> Davidson, R. W., Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>8</sup> Single-spore isolations made by H. C. Greene of the University of Wisconsin.



PLATE II. A. Mycelium grown on malt agar.  $\times 300$ . B. Conidiophores.  $\times 300$ .  
C. Spores. Highly magnified. D. Mycelium in wood.  $\times 225$ .

bilis (Loudon) Forbes), yellow birch (*Betula lutea* Michaux), black spruce (*Picea mariana* (Miller) Britton, Sterns, and Poggenberg), loblolly pine (*Pinus taeda* L.), Douglas fir (*Pseudotsuga taxifolia* (LaMarck) Britton), red oak (*Quercus borealis* Michaux f.), southern cypress (*Taxodium distichum* (L.) Richard), and western hemlock (*Tsuga heterophylla* (Rafinesque) Sargent). Each stick was steamed 30 minutes at atmospheric pressure prior to inoculation. Within 3 weeks after inoculation all of the sticks were stained from a jasper pink to a light jasper red, the same color as the stained specimens from which the fungus was originally isolated. Sticks with a moisture content of 90 to 100 per cent were stained more intensely and more uniformly than sticks with a moisture content of 40 to 50 per cent. The sticks with high moisture content were stained throughout. Microscopic examinations showed only scattered hyphae (Pl. II, D) throughout the wood, but they were somewhat more numerous in the resin ducts and rays.

#### SUMMARY

A fungus isolated from pink-stained southern yellow pine sapwood and heartwood that is morphologically like a fungus isolated from the heartwood of cypress and from oak was found to be capable of producing the same color in the heartwood and sapwood of loblolly pine, yellow birch, cypress, western hemlock, black spruce, silver fir, red oak, and Douglas fir. It has been classified as a species of *Geotrichum*.

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BUREAU OF PLANT INDUSTRY,  
IN COOPERATION WITH  
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#### THE OCCURRENCE OF HELMINTHOSPORIUM TURCICUM IN THE SEED AND GLUMES OF SUDAN GRASS<sup>1</sup>

S. J. P. CHILTON<sup>2</sup>

(Accepted for publication March 1, 1940)

Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) is severely attacked at times by *Helminthosporium turcicum* Pass. How this fungus, which attacks maize and sorghum, overwinters in colder climates is apparently unknown (2), although Eddins (1) states that in Florida it overwinters on débris in the field. Species of *Helminthosporium* have been isolated from maize seed (3, 6, 7, 8), Valleau (8) demonstrating a high percentage of infected seed when a special technique was used. McDonald (4) obtained no evidence of seed infection by *H. turcicum* from

<sup>1</sup> A contribution from the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

<sup>2</sup> The writer wishes to express his gratitude to the agronomists and seed companies who furnished the seed.

maize crops heavily infected with the fungus. Sherbakoff and Mayer (5), however, report it as causing an ear rot, and A. H. Eddins, in a letter to the writer, states that he has isolated the fungus from maize seed. It is the purpose of this paper to report its presence and prevalence in the seed and glumes of Sudan grass.

#### MATERIALS AND METHODS

Fifty-two lots of Sudan grass seed produced in 10 States were used in the studies. Twelve lots were produced in 1939, 34 in 1938, 5 in 1937, and 1 in 1936. The lots from the 1936, 1937, and 1938 crops were tested in the spring of 1939, those produced in 1939, in the fall of the same year. The technique for determining the presence of fungi consisted in separating the seed and glumes, surface sterilization with 95 per cent ethyl alcohol for one minute, 1-1000 aqueous solution of bichloride of mercury (5 minutes for seed and 1 minute for glumes), followed by immersion in a saturated solution of calcium hypochlorite until transferred to plates of potato-dextrose agar. Both seed and glumes were left from 6 to 15 days, after which identifications were made. One hundred and fifty or more seeds and 88 to 200 glumes were tested for each lot. Over 9,000 seeds and 5,000 glumes were plated. In the case of *Helminthosporium turcicum*, inoculations with appropriate checks were made with single-spore cultures on Sudan grass plants and the fungus reisolated to ensure pathogenicity of the isolates. Germination studies of the seed were made on moistened filter paper in sterile Petri dishes.

#### EXPERIMENTAL RESULTS

The data in table 1 show that 21 of the 52 seed lots, or 40 per cent, were infected with *Helminthosporium turcicum*, and one or more infected lots were obtained from 7 of the 10 States. The fungus was demonstrated in the seed and glumes of 16 lots, in the glumes of 3 lots, and in the seed alone in 2 lots. The percentage of infected seed varied from 1 per cent to 20 per cent,

TABLE 1.—*Seed lots of Sudan grass from which Helminthosporium turcicum was isolated at State College, Pennsylvania, in 1939*

Source of seed	Number lots tested	Number lots infected	Number lots with seed and glumes infected	Number lots with glumes alone infected	Number lots with seed alone infected
California . . . . .	11	0			
Georgia . . . . .	2	1	1		
Illinois . . . . .	5	5	3	2	
Kansas . . . . .	2	0			
Nebraska . . . . .	15	9	8	0	1
New Mexico . . . . .	2	1	1		
Oklahoma . . . . .	2	2	1	1	
South Dakota . . . . .	2	1	1		
Texas . . . . .	10	2	1		1
Wisconsin . . . . .	1	0			
	52	21	16	3	2

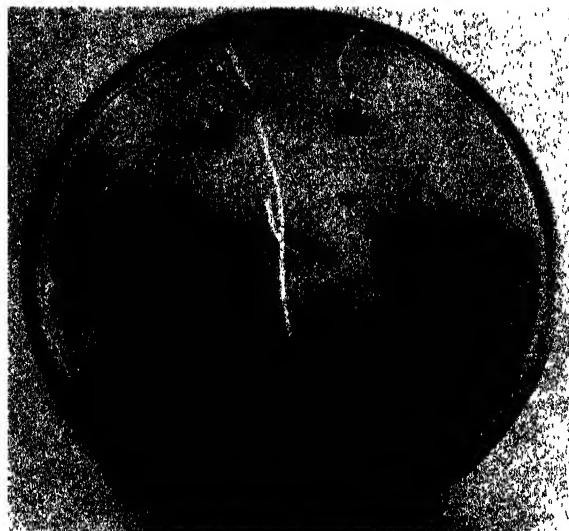


FIG. 1. *Helminthosporium turcicum* emerging from surface sterilized seed of Sudan grass stored two winters.

and of glumes from 1 per cent to over 50 per cent, respectively (Table 2). It is possible that the fungus was present in the other seed lots and was superficial enough to be destroyed by the sterilization technique, or that a random sample was not secured. The fungus was found in the seed or glumes of 4 of the 5 seed lots produced in 1937, indicating that the fungus can remain viable in seed and glumes stored 2 winters. As high as 8 per cent of the seed and 3 per cent of the glumes of these lots yielded *Helminthosporium turcicum*. Figure 1 shows the fungus emerging from seed nearly 2 years old.

TABLE 2.—Frequency distribution of seed lots of Sudan grass with respect to percentage infection of seed and of glumes with *Helminthosporium turcicum*

Materials	Per cent					
	1-5	5.1-10	10.1-15	15.1-20	20.1-50	Over 50
Seed	8	5	3	2	0	0
Glumes	10	5	1	1	1	1

Seed of 2 infected lots was sterilized for 5 minutes, 1 hour, and 3 hours, respectively, in bichloride of mercury solution, the time in alcohol and calcium hypochlorite being the same as previously mentioned. The results (Table 3) show that 1-hour sterilization reduced germination from 90.4 per cent to 87.9 per cent and the percentage of seed from which *Helminthosporium turcicum* emerged from 15.2 per cent to 2.7 per cent. Three hours' sterilization reduced germination to 72.7 per cent and seed from which the fungus emerged to 1.2 per cent. These results indicate the fungus was located primarily, if not wholly, in the seed coat.

TABLE 3.—*Emergence of Helminthosporium tурicum and germination of seed from 8 lots of Sudan grass seed after different periods of sterilization*

Lot	Sterilization time in bichloride of mercury								
	5 minutes			1 hour			3 hours		
	Number seeds	Percentage germination	Percentage infected	Number seeds	Percentage germination	Percentage infected	Number seeds	Percentage germination	Percentage infected
1	202	92.6	18.8	205	91.2	2.9	224	78.7	2.2
2	205	88.3	11.7	201	84.0	2.5	204	71.6	0.0
All	407	90.4	15.2	406	87.9	2.7	428	72.7	1.2

Germination tests of 22 seed lots free of *Helminthosporium turicum* and 13 infected lots indicate that somewhat fewer seeds germinated in infected lots than free ones, the average germination being 83.5 per cent and 75.5 per cent, respectively. The difference is statistically significant.

Other fungi isolated were species of *Alternaria*, *Helminthosporium*, *Acrothecium*, *Oospora*, *Penicillium*, *Fusarium*, *Chaetomium*, and *Phoma*. As high as 70 per cent of the seeds and 52 per cent of the glumes of some lots were found to contain *Alternaria* spp. *Colletotrichum graminicolum* (Ces.) Wilson was found once or twice in several seed lots and in over 50 per cent of the seeds and glumes of one lot from Georgia.

#### SUMMARY

*Helminthosporium turicum* is reported as occurring in the seed and glumes of a high percentage of the Sudan grass seed lots tested. The fungus may remain viable at least 2 winters in both seed and glumes. With respect to the seed, infection is confined primarily, if not wholly, to the seed coat. Germination was reduced somewhat in the case of infected seed in the lots tested.

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## PHYTOPATHOLOGICAL NOTES

*Monilinia Causing a Brown Rot and Blight of the Common Azalea.*—In a previous paper<sup>1</sup> brief reference has been made to a species of *Monilinia*, which is parasitic and pathogenic upon the common azalea or pinxter flower, *Rhododendron roseum* Rehder (= *Rhododendron nudiflorum* (L.) Torr. var. *roseum* (Loisel.) Wiegand). The name *Monilinia Azaleae* has been proposed for this fungus.

The purpose of the present note is to present a technical description of this fungus for reference, pending the appearance of a more extended paper that has been prepared for publication.

The apothecial stage arising from the mummied, overwintered fruits or capsules of the host was collected on the ground beneath the host plant by the writer on various field trips in May 1924, April and May 1925, and May 1927, at several locations near Ithaca, New York.

The monilioid conidial stage appeared upon leaves and young succulent shoots when the host plant was in bloom early in June and the young pseudosclerotia and the conidial stage within and upon the young developing fruits during the latter part of June and in July in the same locations.

Collections of a monilioid conidial stage causing a leaf blight of *Rhododendron canescens* (Michx.) G. Don made at Winterville near Athens, Georgia, in April 1925, by Dr. Julian Miller, and later examined by the writer, appear to be of the same species.

The measurements used here are for fresh living material.

### *Monilinia azaleae*, sp. nov.

Apothecia 1–2 crescentia parva fundamenta ab exteriore pseudosclerotio in mumiificatis fructibus, adulta, 0.83–3.5 cm. in altitudine, stipitata, cyathiformia ad patelliformia, discus cinnamon-brown (R.)<sup>2</sup> ad Prout's brown (R.) ad obscuriore fuscum-atrum, vertens omnino ad atrum ad inferiorem dimidium stiptis et in rhizoideo caessite stipes laevis et gracilis, cylindraceus, attenuatus leviter et puberulus ad inferiore parte 0.5–2.0 mm. latitudine et 0.4–3.0 cm. longitudine; rhizoideus caespitosus praesens, conspicuus, atrofuscus, capilliform, flabelliform radians, ex inferiore parte stiptis; discus aperiens cyathoid et tum infundibuliformis, postea patelliformis 0.2–1.4 cm. diam., margo exilis interdum maturus fissuratus et recurvus; asci cylindracei clavati 178–258 × 11–16.5  $\mu$ , modus 211.5 × 13.2  $\mu$  medianus 213.48 × 13.8  $\mu$  apice rotundo incrassato perforato poro, cuius claudens substantia ope jodi aliquanto cyanescente tingit, octospori; ascospori saepe obliqui et uniseriales in parte superiore asci, saepe subbiserialis, elliptici finibus rotundis, hyalini, continui, saepe cum centrali refringente macula, limites 9–20 × 5–14  $\mu$ , modus 15 × 8.75; medianus 13.88 × 9.48  $\mu$ ; paraphyses abundantes, filiformes, ascis aequilongi, apicibus subclavatis attenuati paulatim ad basem, continui vel uno aut duobus septis ad partem inferiorem, hyalini; ectostroma crescens sub epidermem, maxime in foliis, juvenilibus ramulis et fructibus fructificans cinereum incrementum de conidiis, saepe nervicola in superficie superiore foli et in superficie fructus; conidia (macroconidia) limoniformia continua, hyalina, limites 8.5–19.0 × 5.5–14.5  $\mu$ , modus 11.1 × 8.8  $\mu$ , medianus 12.37 × 9.59  $\mu$ , crescens in longis di- et trichotomis eatenis, adultum parvi, fusiformes disjunctoriae saepe inter conidia; spermatia (microconidia) non observata; pseudosclerotia in aegrotantibus capsulis, adulta complexta loculos fructos hyalinis hyphis incrassatis muris dispositis paliforme in contactii cum vallo pericarpi et dissepi-mentorum placenta et ovules circum data in albo incremento fungi, solidum, compleps

<sup>1</sup> Honey, E. E. North American species of *Monilinia*. I. Occurrence, grouping and life histories. Amer. Jour. Bot. 23: 101, 105, 106. 1936.

<sup>2</sup> (R.) = Ridgway, R. Color standards and color nomenclature. 43 pp., 53 col. pl. Washington, D. C. 1912.

mumificatam capsulam; hab., parasitica in foliis, ramaulis et fructibus *Rhododendroni roseum* Rehder, Enfield Gorge, Ithaca, Tompkins County, New York.

*Apothecia* one to two arising as small fundaments from the outer surface of the pseudosclerotium in mummied fruits, attaining at maturity a height of from 0.83–3.5 cm., stipitate, cyathoid to patelliform, disc cinnamon-brown (R.) to Prout's brown (R.) to a darker brown-black, becoming entirely black toward the lower half of the stipe and on the rhizoidal-tuft; *stipe*, smooth, slender, cylindrical, tapering slightly and somewhat pubescent toward the lower portion, 0.5–2.0 mm. in breadth and 0.4 to 3 cm. in length; *rhizoidal-tuft* present, conspicuous, blackish, capilliform, radiating, somewhat fan-shape from its point of origin on the basal portion of the stipe; *disc* expanding becomes cyathoid, then infundibuliform, later patelliform, from 0.2 to 1.4 cm. in diameter; margin then occasionally cleft at maturity because of a recurring of the disc resulting in radial splitting from the circumference inward;

*Asci* cylindric-clavate, 178–258 × 11–16.5  $\mu$ , mode 211.5 × 13.2  $\mu$ , mean 213.48 × 13.8  $\mu$  with rounded thickened apex perforated by a pore, the closing substance of which stains moderately blue with iodine, 8-spored; *ascospores* commonly arranged obliquely uniseriately in the upper end of the ascus or occasionally subbiserially, elliptical, with rounded ends, hyaline, continuous, commonly with a characteristic-shape central refractive spot, measurements give limits of 9–20 × 5–14  $\mu$ , mode 15 × 8.75, mean 13.88 × 9.48  $\mu$ ; *paraphyses* abundant, filiform, about the same length as the asci, slightly swollen toward the tips, gradually tapering toward the base, nonseptate or with 1 or 2 septa toward the basal region, hyaline.

*Ectostroma* developed beneath the epidermis, particularly on the leaves, the young succulent shoots and fruits, forming an ash-gray coating of the conidial fructification, commonly on the upper surface of the midrib of the leaf and on the surface of the fruits.

*Conidia* (macroconidia) limoniform, continuous hyaline, measurements give limits of 8.5–19 × 5.5–14.5  $\mu$ , mode 11.1 × 8.8  $\mu$ , mean 12.37 × 9.59  $\mu$ , borne in long di-and trichotomously branched chains, at maturity small fusiform disjunctors commonly present between the conidia.

*Spermata* (microconidia) not observed but possibly present in this species.

*Pseudosclerotia* develop in the infected capsules, at maturity, filling the loculi of the immature fruit with a solid mass of thick-walled hyaline hyphae that assumes a more or less palisade-like arrangement at the point of contact with the wall of the pericarp and the dissepiments, the browned and shrivelled remains of the dissepiments, placenta, and the ovules are plainly recognizable embedded in the white fungus growth of the solid pseudosclerotium in the mummied capsule in cross sections, capsules containing pseudosclerotia do not open but fall to the ground, overwinter, and later may give rise to the apothecia.

*Host.* The apothecial stage develops on the fallen mummied fruits of the common azalea, *Rhododendron roseum* Rehder, which have overwintered on the ground in the leaf mold under the shrubs in somewhat moist shaded places during the latter part of April and the first part of May in central New York.

The conidial stage is parasitic upon *Rhododendron roseum* appearing first upon scattering leaves and young succulent shoots when the host is in full bloom, early in June, and common upon the young and developing fruits the latter part of June and July.—

EDWIN E. HONEY, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

*Fruit Stripe of Tomato Caused by a Tobacco Type 1 Virus.*<sup>1</sup>—During the summers of 1936 and 1937, some of the tomato fruit harvested from the experimental field plots at Pullman, Washington, showed chlorotic to necrotic stripes extending from the stem end towards the blossom end (Fig. 1). Cuttings were made from the affected plants and transferred to the glasshouse for study. Transfers<sup>2</sup> from the affected cuttings to benched tomato plants in a comparative test with transfers of tobacco mosaic, potato vein-banding, potato mottle, and combinations of these diseases showed the tomato fruit stripe to be distinctive in symptoms.

#### SYMPTOMS

The distinguishing symptoms of the disease are the stripes on the fruit. They appear first near the stem end as somewhat raised light-green to

<sup>1</sup> Published as Scientific Paper No. 430, College of Agriculture and Agricultural Experiment Station, State College of Washington.

ashen-grey stripes, 1 to 2 mm. in width, radiating from the point of stem attachment for varying distances towards the blossom end of the fruit. As the fruit enlarges the stripes may become broken, somewhat brownish, and sunken (Fig. 1).

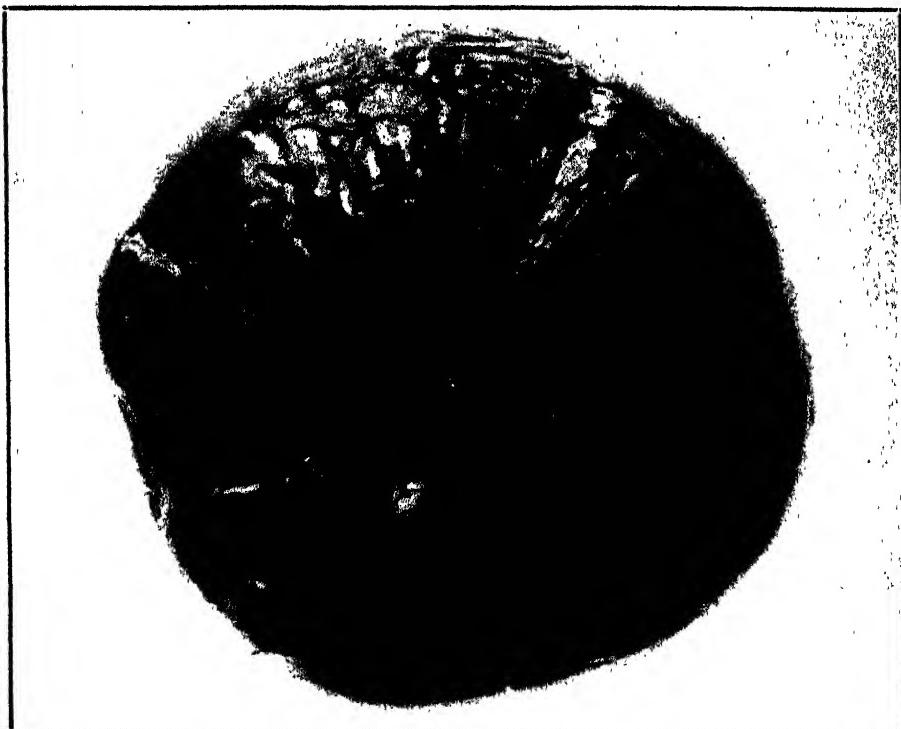


FIG. 1. Tomato fruit showing chlorotic and necrotic stripes radiating from the stem end.

The foliage of affected plants shows a mild mosaic (Fig. 2, C) without necrosis. No necrosis is noted in stems or petioles, as is the case with plants affected with the single virus streak, or the streak disease caused by the combination of the tobacco-mosaic virus and the potato X virus. On tobacco foliage the virus produces only a mild mosaic on the young leaves (Fig. 2, B), which is followed by numerous small chlorotic spots as the leaves grow older (Fig. 2, A).

Combination of the potato X virus and the fruit-stripe virus on tomato plants produced streak symptoms, and on tobacco plants leaf-necrosis symptoms similar to those produced by combining the tobacco mosaic virus and the potato virus in these suspects.

#### CHARACTERISTICS OF THE VIRUS

Inoculations to 10 tomato and 10 tobacco plants in each test showed the fruit-stripe virus to have an incubation period of 12 to 15 days; it was

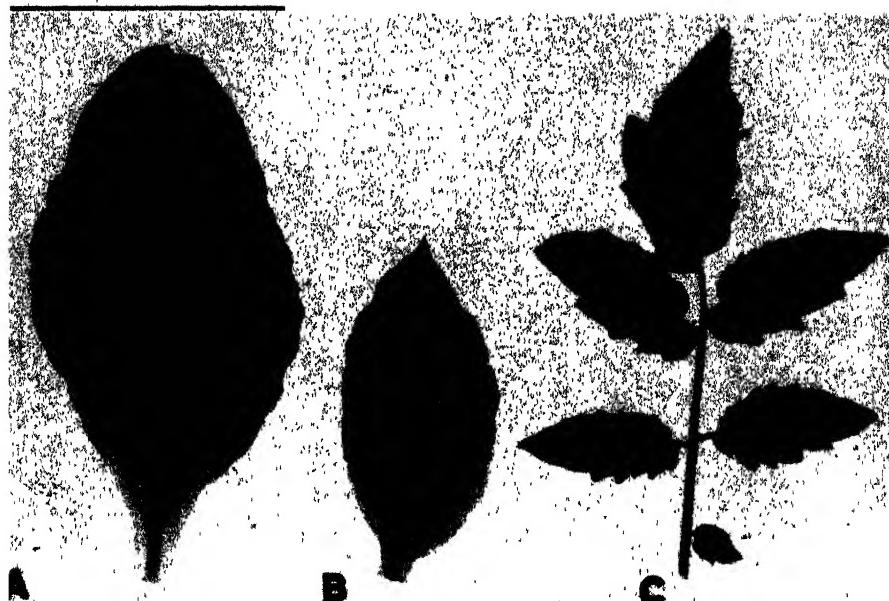


FIG. 2. Effect of tomato fruit-stripe virus. A. Older tobacco leaf. B. Young tobacco leaf. C. Tomato leaf.

inactivated at 90° C. and not at 80° C. for 10-minute exposures; it remained active in 1:1,000,000 dilution with water; and remained active for a period of at least 65 days *in vitro*. The general characteristics of the virus were the same as those exhibited by tobacco virus 1 and, accordingly, it is considered a variant of the tobacco-mosaic virus.—LEON K. JONES, Division of Plant Pathology, Agricultural Experiment Station, The State College of Washington, Pullman, Washington.

*Fumigation Injury of Chrysanthemum.*<sup>1</sup>—It is a common practice in glasshouses to burn Nico-fume powder for the control of aphids. During the growing season, of 26 varieties of chrysanthemums, in a study of the *Verticillium* disease, the house was fumigated with Nico-fume when the buds of many varieties were partially opened. Nico-fume, to fill two 2½-in.

TABLE 1.—*Chrysanthemum varieties subjected to Nico-fume fumigation*

Adrian's Pride	Early Frost	October Frost
Ambassador	Friendly Rival	Pink Mistletoe
Bonaffan	Golden Measure	Pink Treasure
Chadwick	Indianola	Razor
Chieftain	Justrite	Seidewitz
December Beauty	Lustre	Turner
December Glory	Maude Dean	Whittier
Dorothy Turner	Monument	W. H. Waite
Dr. Enguehardt	Oconto	

<sup>1</sup> Published as Scientific Paper No. 431, College of Agriculture and Agricultural Experiment Station, State College of Washington.



FIG. 1. Ring of necrotic floral parts of Whittier blossoms caused by Nico-fume fumigation. Uninjured blossom in center.

pots, was burned in 5500 cu. ft. of glasshouse space in the early evening, and the ventilators remained closed until 7:30 the next morning.

When the Whittier variety opened it was noted that most blossoms showed a ring of brown, dead, floral parts, 2 to 3 inches wide near the base (Fig. 1). None of the flowers of the other 25 varieties (Table 1) showed this injury. A similar planting of the 26 varieties in an adjacent section of the glasshouse was not fumigated and all blossoms were free from the trouble.—LEON K. JONES, Division of Plant Pathology, Agricultural Experiment Station, The State College of Washington, Pullman, Washington.

*Notes on Septoria Scalds of Vetch and Peas in Oregon.<sup>1</sup>*—One of the most important diseases of vetch, particularly *Vicia sativa*, which is an increasingly valuable seed crop in Oregon, is a stem rot or scald and leaf spot caused by *Septoria viciae* West. This fungus causes a purple to vinaceous cortical rot on the lower culm. Spotting and speckling extends up the stems and onto the leaves. After rains in late winter, the spotting coalesces to cause extensive scorching. While the culm injury is confined to cortical cells, the area covered is so extensive that injury is severe and reduction in seed yield is apparent at harvest. Often the fungus does not fruit, making identification uncertain, but in some cases spores are produced in great numbers. Spread of the disease is by spores, which, splashed by rains, cause the severe speckling or scalding.

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published as Technical Paper No. 331 of the Oregon Agricultural Experiment Station with the approval of the Director. Contribution from the Department of Botany.

The fungus has two kinds of pycnospores, macrospores ( $53-71 \times 1.7-2.1 \mu$ ) and microspores ( $3.5-11 \times 1.2-1.5 \mu$ ) (Fig. 1). The macrospores are

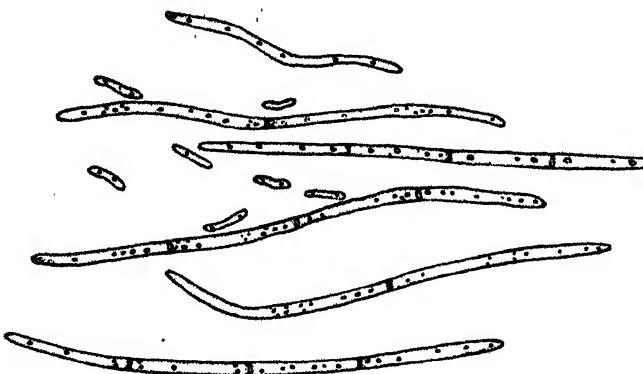


FIG. 1. Pycnospores (macrospores and microspores) of *Septoria viciae* West. on *Vicia sativa*. Granger, Oregon, June 13, 1939.  $\times 1000$ .

straight to curved or slightly sinuous, broadly filiform, mostly 3-septate, with small oil drops in the cells. The microspores are nonseptate and bacillar-shape. The fungus can be assigned to *Septoria viciae* West. Common vetch is very susceptible, Hungarian vetch apparently less so.

A cortical stem scald of Austrian field peas has been observed in Oregon for a number of years. Its symptoms are similar to those of *Septoria* scald on vetch. It apparently is caused by *Septoria pisi* West and, in Western Oregon, is often the sole cause of severe injury to Austrian field peas in early spring. In other cases the fungus occurs with *Ascochyta*.

These diseases should be studied further as they are of economic importance, particularly in the control of seed-crop diseases in Oregon.—RODERICK SPRAGUE, North Dakota College of Agriculture, Fargo, N. D.

## BOOK REVIEW

THOMPSON, HOMER C. *Vegetable Crops.* (3rd ed.) 578 pp., 68 figs., \$5.00. McGraw Hill Book Company, New York and London. 1939.

Although this textbook was prepared primarily for students and others interested mainly in the culture, production, and economics of vegetables, phytopathologists and economic botanists will find it especially valuable as a reference. In it the author, who has spent his entire career specializing in this particular field, has assembled a veritable mine of scientific data on the different phases of the vegetable industry.

The book consists of 27 chapters and is well illustrated with 68 figures, besides numerous tables. It might be said really to be composed of two parts. The first part consists of 15 chapters in which the author discusses the scientific facts and principles involved in the growth of the vegetable from germination of its seed to its harvest and storage or placement on the market. There is treated in these chapters such pertinent subjects as soils and soil preparation, manures, soil-improving crops, seed and seed growing, and methods for the control of diseases and insects. Information, useful not only to the pathologist specializing in the diseases of vegetables, but also to the extension pathologist or, even the general pathologist called upon to diagnose diseases of vegetables.

The second part of the book is composed of 12 chapters in which the various groups of vegetables are discussed individually. For example, in the chapter devoted to perennial crops, such vegetables as asparagus, artichoke, Jerusalem artichoke (*Girasole*), and sea kale are considered. In another chapter, the potheights or greens, spinach, New Zealand spinach, orach, chard, kale, mustard, collards, and dandelions are considered. Other chapters are devoted to cole crops, root crops, bulb crops, and the potato, the sweet potato, beans and peas, solanaceous fruits, the cucurbits or vine crops, and sweet corn.

For each vegetable (at least the important ones) there is given and discussed its history, taxonomy, characteristics, climatic requirements, soil preferences, soil reaction, culture, diseases, and insects, if they are of economic importance.

In this book the author shows that he appreciates the economic importance of diseases and insects in the growing of vegetables, inasmuch as he advises students specializing in olericulture, to schedule during their collegiate careers courses on plant diseases and harmful insects. Furthermore, he stresses the danger of the transmission of diseases and insects into new areas by the indiscriminate shipment of plants.

For each crop the author lists the important diseases and standard control measures. This information assembled under one cover should be especially helpful to vegetable growers who use this book as a reference and are not specialists in the field of phytopathology. Therefore, it is contended that the control measures recommended should be as up-to-date and concise as possible, so as not to be in any way confusing. In some instances, the directions given for controlling some of the diseases are rather incomplete and indefinite or are cited as being effective in a certain section of the country. The user of this book is undoubtedly not so much interested in the effectiveness of treatment in one section as he is in knowing whether he can control or eradicate a disease in his own locality.

In so comprehensive a volume occasional errors and omissions would be expected. In the chapter on the diseases of sweet potatoes the author omits mention of such diseases as soil rot or pox. This disease, which is widely distributed and important, has become very serious in some sweet-potato areas in recent years, so bad in some cases that the yield has been reduced considerably. In some instances it has resulted in the abandonment of any further culture of this vegetable. Reference in the text is made to the monographic study of the diseases of sweet potato by Harter and Weimer but the title or a citation of it is not to be found in the literature cited at the rear of this book.

Inconsistencies appear in the use of authorities for names of seed plants, fungi and insects. Although these are not particularly serious, they may be confusing to the students and others who use this textbook as a reference. For example, the scientific name and authority for cress or garden cress (*Lepidium sativum* L.) is cited, while the authority for the scientific name of chervil or salad chervil (*Anthriscus cerefolium*) occurring on the same page as the preceding vegetable, is omitted. Similarly, some inconsistencies appear in respect to capitalization of specific names. Other small errors and omissions occur throughout the book, but the author will doubtless correct them in his next revision.

—THEODORE T. AYERS, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

## ATTENTION! PLANT PATHOLOGISTS

Your committee on Publicity and Public Relations has been charged with the duty of working out ways and means of bringing before the public, as soon as it becomes available, information concerning the newer developments in the nature and control of plant diseases.

The functions of the committee obviously cannot be carried out without the continued cooperation of the members of the Society. The committee earnestly appeals to all active plant pathologists to send in newsworthy material of their work. These may be reprints of new publications, a copy of the manuscript after acceptance by a scientific journal, or a description of the work designed especially for the committee's use.

From this information, the committee will develop news articles or magazine articles or will transmit the information to one of the several science writers to be syndicated in their publications. In every case the author will be given full credit. The information will not be used without the full consent of the author and of the authorities at his institution, if such consent be necessary. It will be the constant care of the committee that no untrue or distorted interpretation of the facts shall be written by reporters.

The committee will not interfere in any way with any established State, federal or other publicity work.

We would suggest that all available information be sent to the committee-man representing your section of the country or to the chairman if you so desire. Please state if the information can be used immediately or give its approximate date of release.

C. T. GREGORY, *Chairman*  
Committee on Publicity and Public Relations

## SUMMER MEETING OF THE NORTH CENTRAL STATES GROUP OF PHYTOPATHOLOGISTS

The North Central States group of phytopathologists will conduct a summer tour in western Illinois from June 20 to 22. The group will assemble at Quincy, Illinois, on June 20. June 21 will be spent on tree fruit and small fruit diseases near Quincy and on grain diseases in the Illinois River bottom near Jacksonville. The afternoon will be devoted to an inspection of the experimental orchard spraying work at Jerseyville. The night will be spent at the famous Pere Marquette State Park near Grafton.

On June 22, the group will tour the intensive vegetable area in the Mississippi River bottom near East St. Louis where various vegetable and field crop diseases will be seen.

Members of the Society other than those in the North Central States (Michigan, Wisconsin, Minnesota, Iowa, Nebraska, Missouri, Illinois, Indiana and Ohio) who plan to attend the meeting should write Dr. H. W. Anderson for detailed program about May 20.

Committee on Arrangements,  
C. M. TUCKER  
I. H. MELHUS  
H. W. ANDERSON





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